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**A quantitative and qualitative approach
to cuttlefish (*Sepia officinalis*) body
patterning**

Jay Culligan

Submitted for the degree of Doctorate of Philosophy
University of Sussex
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Summary

Cuttlefish are renowned for their ability to quickly alter the colour and texture of their skin, for camouflage and communication. This is due to the presence of thousands of pigment-filled sacs, known as chromatophores, which are distributed across the skin. The chromatophores are innervated by motoneurons, which dilate the chromatophores to create the spots, stripes, and other markings, known as chromatic components. There are 34 recognized chromatic components, and it is an interesting question how cuttlefish coordinate the expression of these components to camouflage and communicate.

The digital age has introduced new, powerful algorithms and methods to tease out subtle features in the coloration patterns, by means of image registration, segmentation, and identification, as well as methods for modeling the underlying control systems. These tools offer major new insights into the mechanisms of visual perception. In addition, powerful techniques have recently been developed that have yet to be applied to this complex visual motor control system. These methods have large potential in helping discover what features between the pattern and the environment are necessary to prevent detection.

Here I present four laboratory experiments, that for the first time use machine learning models, to investigate cuttlefish pattern formation, implementation, and information.

The first two experimental chapters investigate how cuttlefish orchestrate their chromatic components for camouflage patterns, and what strategies they employ on diverse backgrounds. I demonstrate that components are expressed more independently than previously believed, finding that the range of patterns expressed lie on a continuum, allowing us to suggest a revised classification scheme for cuttlefish body patterns. The diversity of patterns seem to imply that a cuttlefish could use its repertoire flexibly to display the maximally cryptic pattern for a given background, however I show that cuttlefish do not in fact select a single (possibly optimal) camouflage pattern, continually alter their appearance on a given background, and that the frequency of change increases in relation to the size of the objects in the environment.

My third chapter investigated the language-like properties of cuttlefish communication using human speech recognition models. From our subset of cuttlefish patterns, I discovered cuttlefish utilize a lexicon of 10 patterns, with language-like properties such as: they obeyed Zipf's law, contained around 1.6 bits per display, and interestingly, while 2 patterns were visually similar, they were displayed in separate contexts. By implementing a regression onto the patterns, I introduce a basic dictionary of cuttlefish terms and their meaning.

From my investigations into cuttlefish intraspecific signaling, I discovered two previously undocumented patterns, used in agonistic encounters between cuttlefish. My final chapter describes these patterns and the contexts they are displayed.

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1. Introduction: A review of camouflage methods

Over the course of evolution animals have employed a wide variety of camouflage techniques to avoid predation, with sophisticated and diverse methods employed to prevent or obscure recognition in each sensory modality (Ruxton 2009). Particular interest lies in visual camouflage, and many studies have either examined how well an animal may prevent detection, or endeavored to determine the qualities that enable an animal to blend with its surroundings. The present review examines the techniques that have been described in the literature for the study of camouflage, and seeks to identify those that are most effective or productive. It should be noted however, that as we consider many of the techniques as tools, where we provide a more broad account the methods and their implementation, in some cases we given only a brief account of how they work.

The need for prey to avoid predators, and for predators to avoid detection, results in strong evolutionary pressure for animals to hide their shape or blend into the background. Camouflage studies have been useful in understanding these evolutionary pressures and adaptations, providing Darwin (1859) and Wallace (1889) with support for their earliest arguments for natural selection. Study of camouflage has extended into many disciplines of science, with vision science, in particular, benefitting from an understanding of how predators and prey adapted to changes in their environment (e.g. Ruxton et al. 2004), especially in cephalopods (Barbarosa et al. 2008, Chiao & Hanlon 2001, Hanlon et al. 2009, Kelman et al. 2008, Mathger et al. 2006, Zylinski et al. 2012). Animals use several types or methods of visual defense, the most common being colours and patterns that prevent detection (crypsis), and accordingly these are the type of camouflage that we concentrate on here. Other forms of defense include masquerade where the animal resembles an uninteresting object, and aposematism where bright colours warn predators of the animal's (real or faked) unpalatability.

Cryptic camouflage is often characterized as having two main visual strategies: background matching and disruptive coloration. In background matching the body has similar patterns, or reflectance properties to the background. Disruptive coloration adds to this by including false edges and boundaries from contrasting colours, which serves to disrupt the outline or shape of the animal (Stevens and Merilaita 2009). Recent research has examined

how coloration patterns are related to an animal's ecology and environment; for example, Allen and colleagues (2010) found that, in felids, pattern complexity and irregularity are related to habitat, and that camouflage adapts to ecology over relatively short time scales.

Often body patterns need to provide defense against multiple predator species (Osorio and Cuthill 2013). Considering the range of coloration and body patterns across the breadth of the natural environment, and their effectiveness measured against the human visual system, suggests that other species' visual systems may share similar principles of perception (Stevens 2007, Osorio and Cuthill 2013). Nevertheless, over-reliance on human vision can generate false (evolutionary) hypotheses, notably about colour in the animal world (Bennett 1994), because of the diversity of colour vision systems. For example, bees have three colour channels and their trichromatic vision extends into the ultra violet (UV), while birds have four channels of spectral sensitivity, one of which is UV sensitive (Osorio and Cuthill 2013). Indeed, birds arguably possess one of the best-equipped visual systems of any animal, especially beneficial for foraging and during mate selection (Bennett & Thery, 2007). However, similarities between visual systems are thought to lie beyond colour and are also found in texture perception and spatial detection, but by comparison with colour vision our knowledge is limited and we should be cautious about generalization. Finally, polarization, which may be significant in camouflage and display to other species (especially outside vertebrates), is functionally invisible to humans.

Our working assumption is that natural environments in which visual systems evolve can be characterized by their statistical structure, and that finding the relevant statistics will give insight into both camouflage design and visual mechanisms. Specifically, we review the aspects of visual perception that are generally considered to be important for figure-ground segregation, beginning with visual texture (Julesz, 1971), and then low level image statistics (e.g., together with first-order (distribution of pixel intensity values) and second-order (power spectra) statistics of natural images. We also consider sampling approaches (e.g. Endler, 1978), and what might be called 'pragmatic approaches' to the description of visual stimuli, potentially involving higher order statistics that are difficult to measure, but nevertheless may provide important information about an image.

1.1 Statistical analyses

Historical roots

A pioneer in our subject was Charles Darwin's grandfather, Erasmus Darwin, who in 1794, wrote about animal coloration and pattern as camouflage (Cuthill & Troschianko 2009). Even if the principles behind camouflage were not understood, its function was clear. It took a further hundred years before Abbott Thayer and Hugh Cott introduced concepts and theories that aimed to explain in functional terms the diversity of colours, stripes, and spots in animal patterns (Cott 1940). Thayer and Cott, both artists and writers (Cott was also an academic zoologist), independently pioneered the early theories and definitions we still use to categorise camouflage. Cuthill and Troschianko (2009) give an extensive review of the contribution of the early pioneers in the study of camouflage. Thayer, Cott, and their successors relied heavily on descriptions and illustrations to convey their ideas. Only since about 1980 have sophisticated equipment such as spectrometers, cameras and similar devices, along with computer based analysis of image data, been used to support the investigation of camouflage.

The impact of methods to examine more complex patterns such the use of body pattern transections (Endler 1978; reviewed in section 3.2.1 below) helped shape future experiments, and opened up opportunities to study and understand the role of complex patterns in camouflage. This, together with the revolution in digital imaging that followed shortly afterwards, provided technical advances that have enabled researchers to explore new relationships between the perceiver, the pattern, and the background. Digital image analysis removed the necessity of having direct measurements of the patterns and the environment, allowing researchers to take photographs in natural settings, rather than in the laboratory alone.

Digital images and order statistics

Light entering the eye forms a retinal image containing information about colour and brightness; over time the pattern of light changes as things in the world move relative to the eye (Victor and Conte 1995). The early visual system in humans attempts to analyse the patterns of light entering the eye in order to recover the shape, appearance, and motion of objects in the world. Computer or machine vision attempts similar analyses, simulating human and other animal perceptual systems, and how they might examine their world. Computational models can also be created to simulate how camouflage patterns might work,

from light hitting the cells of the retina through to higher level processing (Caelli et al., 1978). Digital image analysis, supported by increasingly powerful computers and sophisticated algorithms, provides a means to analyse the relationship between the pattern and its background. These technical advances present opportunities for researchers to explore more complex patterns, examining the relationships between various visual systems and body patterns.

A digital image is a data set, where the light incident upon the pixels in a camera's sensor is transformed through sampling and quantization to a numerical array. From these digitized images, measurements can be taken, statistics derived and inferences made. The aim is to find regularities important for vision and cognition, of course, some measurements are more interesting than others, and it has, in practice, proven remarkably difficult to identify measures that best characterize the appearance of an image to the human eye, or indeed the design of camouflage.

According to reasonable principles of parsimony and in accordance with understanding of the neural and psychophysical mechanisms of vision, regularities of natural images have been described by statistics of a particular 'order', starting from the lowest order. First-order statistics are concerned with the probability distributions of pixel values across an entire image. Second order statistics on the other hand refer to relations between pairs of pixels, which are captured by the autocorrelation function which measures of the relationship between pixels as a function of their distance (Field, 1989), and equivalently by the Fourier power spectrum. Higher-order statistics depend upon the relationship between three or more neighbouring points in an image (Victor and Conte 1995; Srinivasan and Shobha 2008). The higher the number of pixels taken into account (nth order), the higher the degrees of freedom and the complexity of the analysis (Julesz 1975). These statistics provide important information about images, and hence for understanding the visual system. Within this large subject we look at how image statistics have been applied to the evaluation of animal coloration patterns and camouflage.

1.2 First order statistics

Spectrophotometry, spectroradiometry, and polarization

Spectrophotometry, spectroradiometry, and polarization microscopy reflectance allow us to characterize the properties of a single point in an image (Brady et al. 2013). Due to the

similarity in matching a single attribute (e.g. colour or polarity) between the organism and background, these methodologies have been grouped together.

The original evaluations of camouflage coloration utilized colour-matching techniques to measure how closely an animal's body pattern related to the whole background or its components. The methods compared attributes such as coloration, brightness, reflectance, polarization, or radiance (Norris and Lowe 1964; Lillywhite et al. 1977; Donnelly and Dill 1984; Stuart-Fox et al. 2004; Stuart-Fox and Moussalli 2009). In other words, these first order measurements specify for each attribute the difference between the object and its background (or another object).

An exemplary study of this kind is by Stuart-Fox and co-workers (2004) who examined background matching in populations of dragon lizards in the *Ctenophorus decresii* species complex, which is regionally polymorphic and sexually dimorphic in its coloration patterns. They used a spectrophotometer to measure the reflectance spectrum of six regions on each lizard, taking the mean of three measurements in each region, comparing this to the irradiance and reflectance of the whole background. The spectral reflectance measured both the chromatic (hue and colour saturation) and achromatic (brightness) contrast between the animals and background for blue tit (*Parus caeruleus*) eyes, as a representative of the vision of potential avian predators. Colour contrasts were calculated using the model of Vorobyev *et al.* (1998).

Stuart-Fox and co-workers found that both chromatic and achromatic contrast differences between sexes and populations vary between each body region. Regions exposed to avian predators more closely resembled the background than non-exposed regions, and females matched their backgrounds better than males. One member of the species complex, *C. vadrappae*, was statistically more cryptic to its natural habitat than the others. The evidence showed that the population differences could be attributed to crypsis playing a substantial role in the evolution of colour variation, and hence that colour pattern is under selection within the *Ctenophorus decresii* group.

Hyperspectral imaging

Hyperspectral imaging is a powerful tool in collecting and processing information from across the electromagnetic spectrum. It has been used to identify objects, materials, or detect processes in a wide range of disciplines (Gowen et al., 2007; Lu and Fei, 2014). While the human visual system divides the spectrum into three bands (the red, blue, and green

cones), multispectral images can divide the spectrum into many bands. These bands can be fine-tuned and extend beyond the visible spectrum. This allows researchers to examine the images with respect to predator visual capabilities.

Hyperspectral imaging breaks the electromagnetic spectrum into frequency bands, so the entire image is recorded at each wavelength, to produce a datacube of digital images. The x and y coordinates for pixels correspond to those in a normal (achromatic) image, and the z-axis specifies wavelength. While hyperspectral imaging could be applied to evaluate 2nd or higher order spatio-chromatic statistics, the only application for camouflage (to my knowledge) compared first-order attributes (Chiao *et al.* 2011).

Chiao and coworkers (2011) utilized hyperspectral imaging to see how depth affected the contrast of cuttlefish patterns to di- and trichromatic fish vision. Cuttlefish can rapidly alter their body colour, against a wide range of backgrounds for camouflage. Using a hyperspectral camera, they photographed the common cuttlefish (*Sepia officinalis*) against several backgrounds. Combining the quantum catch images from the hypothetical predator's perspective created the di- and trichromatic images. To isolate the colour (hue) information, the luminance information was removed, generating an isoluminant chromatic image.

They discovered that most features were washed out and the overall contrast was reduced. To further characterize the chromatic discriminability space of di- and trichromatic fish, they determined that small fractions of just-noticeable differences (JND) between the animal and background were small and randomly distributed— suggesting cuttlefish possess good colour-matching capabilities. They found that as the water depth increases, the chromatic information reduces, making visual detection more difficult

RGB colour measurement

Digital cameras possess the capability to interpret a wide range of colours for images. A colour is defined by the combination of the red, green, and blue pixel values, typically based on a scale of 0-255, allowing for 16.7 million colours (Stevens et al. 2007; although most modern cameras have a larger range). Computers, digital cameras, and televisions have long used this method of colour combination to create visual displays. Software is available to take the red, green, and blue colour measurements from a given photo, albeit with limitations imposed by the camera's automatic settings that need to be adjusted properly (Stevens et al. 2007).

Clarke and Schulter (2011) compared the body patterns in three closely related stickleback species (*Gasterosteus aculeatus*) to see how evolution developed their colour-changing capability. These closely related species spend their lives in either the benthic (bottom-dwelling) or limnetic (open water) zones of the water column, feeding in the littoral or pelagic zones, respectively. Spectrometric recordings of the natural sidewelling irradiance (to account for upwelling and downwelling light) from the pelagic and littoral zones in Paxton lake identified the range of wavelengths associated with each stickleback species preferred habitat. Backgrounds were created using spectrometry ratings of their ‘littoral’ colour background and ‘pelagic’ colour background to simulate the natural environment.

Sticklebacks were placed in cups containing a littoral or pelagic background and photographed every 20 seconds with an X-Rite/GretagMacbeth Mini ColourChecker chart (X-Rite Inc.) next to the recording cup. The colourchecker chart consists of 24 colour blocks used to assess the RGB values of the stickleback fish in colour or greyscale values. This chart enables the researcher to interpret the colour values in the picture without the limitations imposed by the camera. The RGB values of a circular portion of the background and the dorsal region of the stickleback (circles of equal size) were averaged. R:B, B:G, and R:G ratios of the background and dorsal region were examined for differences between each picture in the time series. Consistency within species was determined using intraclass correlation coefficient.

Neither fish differed in the pelagic background; however benthic fish match the littoral background better than the limnetics. Limnetics deviated significantly more than benthics from the littoral background colour R:B and G:B ratios. No differences were noticed in the R:G ratios.

First order review

The studies described above utilize methods that take the mean value of the animal’s skin, background, or pixels to determine how well the cryptic coloration patterns match the background. While some methods, like the RGB colour measurement, can be used to study second and higher-order approximations, the literature has yet to utilise these methods to do so. Even so, these methods are useful for the background matching coloration patterns, but lack the ability to test the disruptive components, which use higher order statistics to prevent recognition.

First order approximations are especially useful for uniformly coloured animals, say, for pelagic marine species where, because the water column does not provide high variation in colour or spatial scale, higher order approximations are unnecessary. Another example is reflective camouflage with mirror-like plates, which relies more on first-order approximations to gauge the angle of observation and level of contrast between the animal's outline and the reflected light (Johnsen and Sosik 2003, Johnsen et al. 2014).

Naturally such analyses cannot cope with non-uniform spots, stripes, and other patterns that are present in many camouflage patterns. Matching the chromatic similarities provides only a small insight into the camouflage capabilities of the pattern in question. What's also needed is a way to compare the complex pattern as a whole.

1.3 Second order statistics (standard deviation) and higher order statistics

Body pattern transections

Natural environments encompass a wide range of colours, patterns, and textures. Endler (1978), a pioneer in camouflage studies, proposed that an animal needed to match an individual sample of the background to effectively blend in. To accomplish this, he took measurements of body patterns and coded them into a pixel ratio for brightness and compared the patterns to backgrounds to examine how well an animal blended with their preferred habitat.

Endler (1978) used body pattern coloration transects to measure complex body pattern camouflage, by attempting to relate the variation and geometry in the pattern to a random sample from its environment. In Endler's (1978) method, various transects are made across the pattern, with the colours from all transects are scaled on a range of 0-100 by brightness standards. An edge boundary is classified by taking adjacent pixels configured with respect to the minimum resolution of a predator's visual capabilities (Figure 1.1) with the mean differences between points used to create a threshold. Comparisons are made from the means of the product-moment correlation coefficient between the pairs of numbers in each class (e.g. colour, size, brightness). The result gives values of the colour patches on a pattern or background and allows it to fit to a curve, giving the probability a spot could be drawn from the background at random (Endler 1984).

Shreeve (1990) utilized Endler's method to measure the camouflage capabilities in *Hipparchia semele* butterflies in six microhabitats (sand, lichen, dry vegetation, litter, live vegetation, and root mats), and two postures (unexposed forewing and exposed forewing). Photographs of butterflies and their respective backgrounds were taken using a camera from a short distance (0.5 m), and the images subsequently gray-scaled. Random transects of the background (approx. 20 m of the background) were taken, and measured the total amount of each of the ten grey shades, the size of patches of each grey shade, the total size of patches independent of grey shade class and the number of contrast changes between adjacent patches greater than one grey shade in magnitude. For each butterfly, five transects of the hindwing or three transects of the forewing underside (depending on posture) were taken. Indices of crypsis were calculated by comparing each of the variables for the wing with those for each of the background types using the Pearson product moment correlation coefficient defined by Endler (1984). Correlation coefficients vary between +1, when crypsis is perfect, to -1, complete conspicuousness.

Males and females differed on which backgrounds they were most cryptic. Shreeve discovered positive correlation coefficients for all backgrounds except sand backgrounds, indicating good background matching. Postural differences found significantly higher background matching when the forewing coloration was hidden compared to exposed.

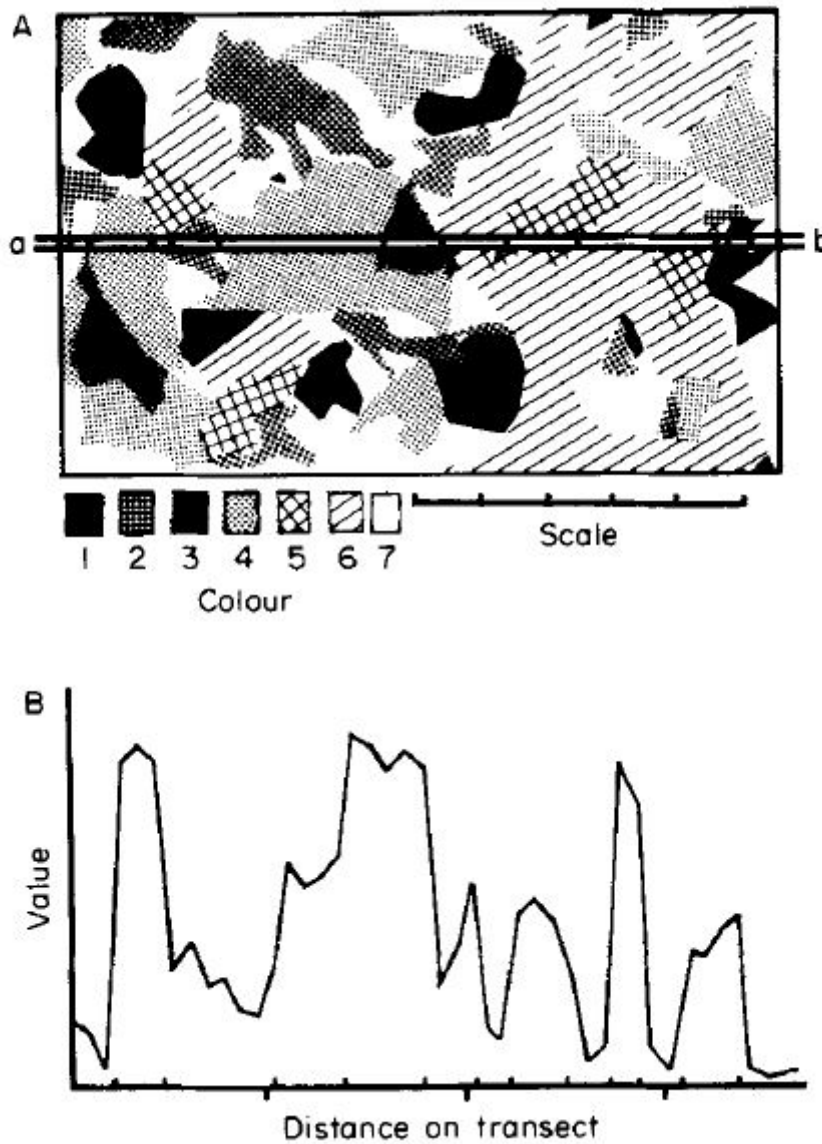


Figure 1.1: Example of body pattern transect from Endler's (1984) transect methodology. A: The original texture with the transect (horizontal line going from a-b) overlying the pattern under analysis. B: The colour intensity (y-axis) over distance (x-axis) of the transect (left to right).

Fourier Analysis

Although there appears to be no universally agreed definition of ‘texture’, it is generally taken to describe what a surface/image looks like in terms of its features, such as, fine or coarse, smooth or irregular, uniform or non-uniform etc. Given this, albeit informal, definition of texture and the description of first order statistics, it is straightforward to understand how regions of an image can be analysed for similarities and differences that can be useful for identifying image features. More specifically with regard to camouflage, first-order methods are useful, for example, in taking the mean value of the animal’s skin, background, or random pixels, to compare how specific regions of a pattern colour-match to specific regions of the background; this is useful for the background matching coloration patterns, but can do little to test disruptive components.

Notwithstanding the positive results identified in the first order studies, the primary issue with these analyses lies in their inability to cope with non-uniform displays; organisms possess myriad spots, stripes and other pattern characteristics where matching the chromatic similarities provides only a limited insight into the camouflage capabilities of those patterns. What is also needed is a way to compare the complex pattern as a whole. The power spectrum is a measure of the strength of energy (power) variations as a function of frequency (f) across an image, and is also a measure of second order statistics; natural scenes are known to show consistent behaviour where power reduces as a function of frequency by a factor of approximately $1/f^2$ (Field, 1987). The power spectrum is the Fourier transform of the autocorrelation function and Fourier analysis can provide important information about an image, providing clues about visual coding (Field, 1989, 1987). Fourier analysis refers to a group of mathematical techniques based on decomposing signals into sinusoids.

The technique used for digital signals and therefore images, is the discrete Fourier transform (DFT). Due to sinusoidal fidelity (Smith 1997), the output signal in the frequency domain is simpler than the input signal in the time or spatial domain, for example, convolution becomes multiplication in the frequency domain, which is a simpler operation that leads to Fast Fourier Transform (FFT) convolution and other techniques. Fourier analysis is used in a similar way for image processing as for other types of signal processing, the main difference being that the information contained in images is not ‘naturally’ encoded in the frequency domain. In other words, the Fourier transform of an image results in moving readily understandable information, in the spatial domain, into a form that is much harder to understand, in the frequency domain (high frequencies give fine detail in an image, whereas

low frequencies give coarser, more global, features). Nevertheless, Fourier based image analysis has useful properties such as the speed improvement when searching for a large pattern within a larger image.

Examining spatial frequency characteristics can help provide important clues to understanding the visual systems across a range of animal visual systems; such analyses are considered important in understanding the physiological underpinnings in the neuroanatomical basis for texture discrimination in the visual pathways, where it is thought that neurons use receptive fields that act as filters for various frequencies (Julesz 1975; Shapley and Lennie 1985; Kiltie and Laine 1992). To illustrate with a camouflage example: the Australian giant cuttlefish (*Sepia apama*), like other cephalopods, is known for its ability to rapidly switch between body patterns. Based on the hypothesis that cryptic patterns will match the intensity and frequency of the background, while signaling patterns (e.g. zebra patterns for male-male aggression) will differ, Zylinski and colleagues (2011) examined the power spectra of body patterns and compared the patterns with natural backgrounds in the wild (Figure 1.2).

While, in essence, Zylinski and colleagues (2011) results showed that the camouflaged patterns blended with the backgrounds more easily and that the signaling patterns differed from the background to make the signal more prominent, correlation and convolution based on FFT has limitations, particularly for the study of camouflage. Significantly, the pattern used for searching must be the same size and orientation as areas being searched for in the image, which is problematic if relative scales are unknown. Scale-invariant feature transform (SIFT; Lowe, 2004) is an algorithm in computer vision to detect and describe local features in images, regardless of the image scale or the level of noise and illumination..

The basic idea of SIFT is that a set of points describing a feature of some object (acquired from a training image) can be used for the identification of that object in a target image. In other words, SIFT characterises objects through feature descriptions, where interesting points on an object (e.g. high-contrast regions such as edges) are classified through the training images. An important characteristic of these training images is that it should allow SIFT to recognise the feature descriptions at varying orientations, scales, noise, and illumination. For example, if the corners of an object can be identified, given their relative spacing, they could be used to identify the object in a different setting (Lowe 2004;

Harris & Stephens, 1988). Once SIFT has been trained on an image set, it scans new images for similar feature descriptions and recognises the object based on the euclidean distance to the trained feature descriptions. This method has shown to respond similarly to neurons in the primate inferior temporal cortex (Lowe 2004). Thanks to this method, SIFT is an excellent tool to tease which features are most responsible for object recognition and has been used successfully to identify the patterns on birds eggs and the features that constitute a recognisable signature (Stoddard, Kilner & Town, 2014). There are many variations of SIFT developed since its original conception, however, to the author's knowledge, none have been used to test camouflage efficacy in any meaningful way.

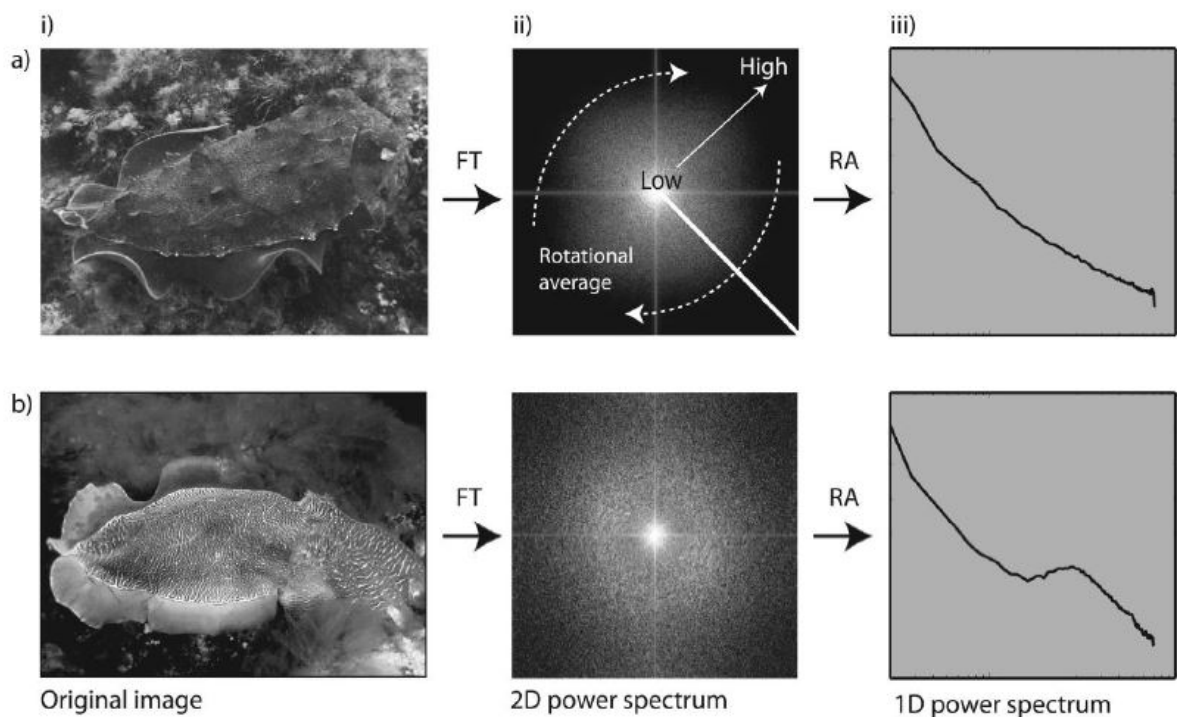


Figure 1.2: A: i) An image of a cuttlefish camouflaging to its environment. ii) The camouflaged image is transformed into the Fourier domain (FT) and (iii) the power spectra of the image obtained. B: i) An image of a cuttlefish displaying a social signal. ii) The social pattern is converted into the Fourier domain (FT) and iii) the power spectra obtained. Observe the power spectra different between the social and camouflage patterns, indicating social patterns do not match the power of their environment to stand out.

Granularity analysis

In the field of digital signal processing, signals can be measured, filtered, or compressed to obtain certain characteristics. Visual information falls in this category when images are transformed in the Fourier domain to examine the various frequencies of a signal. Using filter banks, images can be examined at specific spatial properties (i.e. the low and

high spatial frequencies to show what information is present at each spatial frequency. After the filtered image data has been deconstructed, analysed, and/or manipulated, the image data can be reconstructed into the original image.

Cuttlefish can rapidly change the colour and texture of their bodies, making them excellent subjects to study different background matching and disruptive patterns. Barbosa and coworkers (2008) examined cuttlefish patterns in response to changes in contrast and spatial scale of their backgrounds. Research shows cuttlefish use three broad categories of patterns, originally defined by Cott (1940): uniform, mottle, and disruptive. Cuttlefish display one of the three pattern categories depending on the characteristics of their environment, and each pattern attempts to match the background based on those characteristics.

Cuttlefish were placed on backgrounds with varying spatial scale and contrast. Images were taken and the cuttlefish were digitally cut out from the background. These cut outs were standardized to a cuttlefish template and passed through six, octave-wide, isotropic, ideal filters (i.e. each image was passed through an edge detector at various spatial frequencies to gather edges from the top-bottom, side-to-side, and orthogonal orientations).. Each of the six filters acts as a band pass filter of the spatial characteristics of the pattern, with each band summarized to extract the variance of the squared pixel values, providing a single value of the total power in the image. These six values for each image provided a granularity spectrum for each cuttlefish pattern. Comparing the granularity spectrums showed that each of the three pattern categories (uniform, mottle, or disruptive) displayed unique granularity spectra that could be related to the spatial characteristics of the respective pattern. For example, disruptive patterns with large squares and stripes elicited higher values on lower frequency bands than mottle or uniform patterns. In other words, the granularity analysis enabled researchers to find pattern signatures for each pattern type based on the spatial scale of the patterns.

Visibility Ratio

Digital image analysis poses an issue with how to address depth in a 2D image. Binocular vision uses depth to tease out the animal from its background, enhancing the outline of the object. This means that the object's outline must blend well enough with the background not to be noticed by the visual system. Concealing the contours becomes the principle concern. In this method, Lovell and coworkers (2013) sought to examine camouflage by examining the ratio of background contour to the contours of quail's eggs.

Eggs are an excellent source of nutrition and more easily obtainable than mobile, defensive prey. Oviparous animals have developed strategies to reduce predation in the nest through defending the nest or to develop camouflage patterns to help them blend with the environment. The ground-nesting Japanese quail (*Coturnix japonica*) utilizes the latter strategy to keep their eggs safe. In their natural habitat, a female may select one of several microhabitats which to nest. The eggs possess dark patches on the shell, referred to as maculation and females vary in the amount of maculation on their eggs. They investigated whether females laid eggs on backgrounds where the degree of maculation better matched the substrate. In other words, do the birds lay eggs on substrates that best camouflage the egg?

Female quails were offered four differently coloured backgrounds to lay their eggs. Once laid, each egg was photographed twice: once on the background and another on a black card, both times with a colour chart in perspective. The colour chart normalizes the mean chromacity values to remove illuminance variation. These images were converted from RAW RGB values to XYZ colour space to CIELAB colour space [a form of colour space developed by the International Commition on Illumination (CIE) to provide a perceptually equal, where the Euclidian distance between two colours is strongly correlated with human visual perception (Tkalcic and Tasic 2003)]. CIELAB colour space was used because the values are perceptually uniform, allowing any changes in luminance or other values to change similarly. This allowed Lovell and coworkers to estimate chromatic perceptual differences using 3D coordinates and to calculate the Euclidean difference between 2 CIELAB values.

In order to find the substrate the eggs best camouflaged, they needed to create a mask of the egg to overlay on the various substrates, rather than taking images of each egg on each substrate. They took an RGB mask in Photoshop to create an artificially constructed egg image – a chimeric image of the egg. They copied the parts of the egg onto the central area of each substrate image. This allowed them to match the egg maculation to the background using k-means clustering. Following the automatic segregation of the egg and background, they calculated the mean CIELAB values for the egg maculae (i.e. spots), egg background, and the substrate. Taking the chromacity of each region allowed them to compare the Euclidean distances between each category in a psychophysical colour space (Vorobyev & Osorio 1998). This allowed them to compare the colours between the egg regions (maculation and background) to the substrate.

Crypsis was determined based on the ratio of contour detected between the contours of the egg versus the substrate to calculate the visible ratio (VisRat). They used a computer-vision edge-detection algorithm (Canny edge-detector) to detect the contours. The edge detector was tuned to the visual capabilities of a predator, but considering the wide range of predators and their visual capabilities, they opted for a relatively simple model to detect edges. Contour pixels were scored as part of the egg if they were in an area near the edge of the egg mask (4 pixels into the mask and 8 pixels beyond the mask; equivalent to a range of 1mm). Contour pixels were scored as part of the substrate if they were outside the egg mask and beyond the 1mm egg boundary area. Assuming that any evolved visual system would take the most informative cues, they used the channel with the highest VisRat score to be the metric for egg visibility.

Their initial analyses discovered that the colour of the maculation allowed for best camouflage, but not the egg background colour. Therefore, the quails seemed to choose those backgrounds that matched maculate colour, rather than the background colour of the egg. The eggs varied in their maculation, so they divided the eggs into 4 categories based on the percent of maculation on the egg. They found that eggs with higher maculation had higher contrast with the egg background than eggs with less maculation. The eggs that were laid with lower maculation benefitted from a mixed-strategy, selecting backgrounds that matched the maculation colour or background colour.

Wavelet Analysis

Signals are described as being stationary or non-stationary. Non-stationary signals fluctuate and change their shape, resulting in changes of the mean and variance over time and space (Hammond and White 1995). In contrast, stationary signals maintain their mean and variance. The Fourier analyses described earlier are well-suited to describing stationary signals, and utilize the short-term Fourier transform (hereafter referred to as the STFT) to deal with non-stationary signals. STFT cannot reliably examine non-stationary signals due to the equally spaced windows it places over the signal (Figure 1.3) (Kiltie and Laine 1992; Merry et al. 2005). High frequency components occur in short bursts and are captured using short-windows, while low-frequency components occur throughout the signal and are best captured using wider-windows (Merry et al. 2005). STFT must therefore make accommodations in the window size to capture the variation between high and low frequency components as it is restricted by the equally spaced window sections of a given signal (Kiltie and Laine 1992). This complication is handled through wavelet analysis.

Wavelets are useful for obtaining proper signal resolution for frequency and time simultaneously. The method accomplishes this by analysing a signal at short and long windows to capture the high and low frequencies at differing resolutions (Figure 1.3).

Wavelets utilize an oscillatory wave function which is bounded in time and space (i.e. the wavelet function), rather than a continuous sine-function, to capture the different frequencies. The wavelet function, also referred to as the mother wavelet, is applied to a signal in various time intervals. By translating and dilating the mother wavelet to the signal, the time (translating factor) and frequency (dilating factor) can be graphed (Kiltie and Laine 1992; Merry et al. 2005). Dilating and contracting the scale, changes the shape of the window to allow the capture of high and low frequency resolutions in the signal. Shifting the translation over the course of the signal extracts the time for the respected frequency. Filter banks may be used in the dilation and contraction of the signal to separate frequency bands in a signal for applications of high and low pass filters, downsampling the data, and orientation selection for each of the filters to an image (Kiltie and Laine 1992; He et al. 2007). Similar to the Fourier transformations, the inverse wavelet function allows the signal to be reconstructed. For a full review on the similarities, differences, and applications of Fourier analyses and wavelet analyses, refer to (Merry et al. 2005).

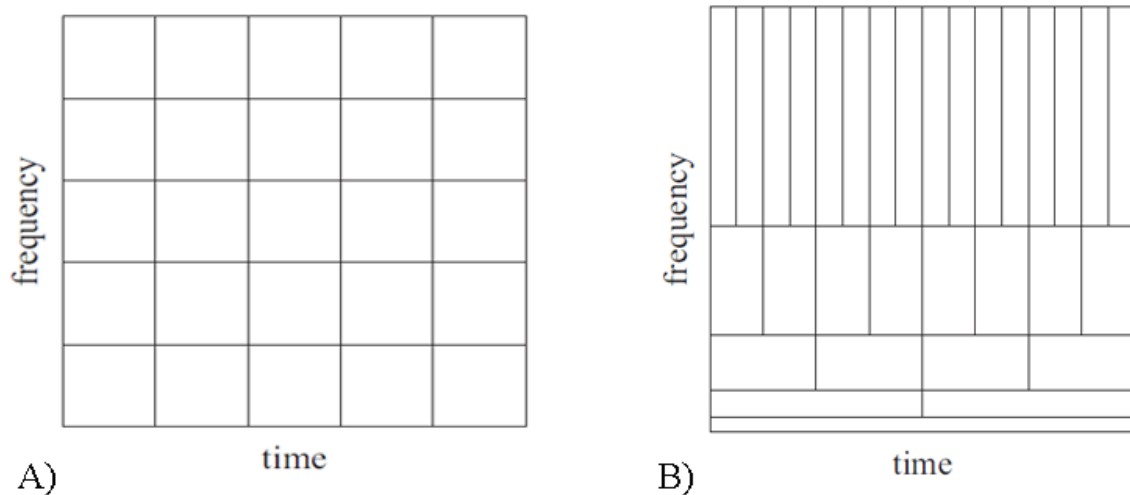


Figure 1.3: A) A graph showing the short-term Fourier transform (STFT) and B) wavelet windows. The STFT windows are equally sized across each frequency, whereas the wavelet windows are scaled to capture different rates of low and high frequency elements in an image.

Colour pattern geometry

Predators and prey rarely examine a single aspect of a scene. A visual scene can hardly be viewed as a still image. The eyes scan the scene, seeking salient cues that may reveal a predator or prey item. Scanning doesn't allow high-resolution imagery, but takes broader aspects of a visual scene into consideration. Accordingly, Endler (2012) proposed a novel method to compare the elements of an animal pattern to the background with consideration to eyes traversing a scene. Colour patches in a visual scene contain broad information on the colour and luminance. As the eyes scan the scene, they attempt to detect changes between adjacent colour patches, with high differences between adjacent colour patches indicating the presence of an edge. In this way, predators and prey can attempt to reduce the difference between colour patches.

This methodology is useful for comparisons between animal patterns as well as for camouflage. For example, comparisons between two patterns can help determine whether aposematic mimics are visually similar to their unpalatable counterparts; but the method can also be useful to examine whether a pattern effectively cryptic. We will discuss the specifics of the methodology that relate to comparing an animal to the background, however, Endler (2012) provides a full review of the methods.

To determine whether two adjacent colour patches are distinguishable, Endler proposes a five step method to compare adjacency statistics: 1) Collect data in a conventional colour pattern analysis; 2) Place the data into a data image; 3) Classify the data; 4) Put the image in a zone map; and 5) Compare the adjacency statistics.

Once the data has been collected, the data image needs to be constructed in one of four possible methods. The first method requires gaining the reflectance spectra tuned to the photoreceptor outputs. This requires dense sampling of the object or image. If the background is included, use radiance rather than reflectance spectra. The second method utilizes RGB/RGBU images from a calibrated camera tuned to estimate the photoreceptor outputs of the visual system in question with the colour and luminance channels separated. Method 3 also utilizes RGB/RGBU digital images as method 2, but doesn't require a calibrated camera. Even without the vision of the perceiver, the data can still yield useful information about colour-pattern spaces. Method 4 uses histological methods to map the patterns of cell types that produce colour in each patch. All methods should account for the visual acuity and viewing distance of the animal pattern.

To classify the data, we need to determine how many colour and luminance classes are present. First, the image data needs to be converted into an image matrix. Next, we determine the number of colour and luminance classes separated by at least one just noticeable difference (JND) in colour, luminance, or both. The number of classes is determined through the scale of variation among samples, or by clustering the sample (e.g. k-means clustering).

Once the number of colour and luminance classes is defined, we construct a zone map. This means that the classified pixels on the image are placed into a zone map where there is a class number for each pixel, instead of the original pixel. Each class number relates to the colour or luminance class from the clustering or scale of variation in the data classification section.

For the adjacency analysis, each point on the zone map corresponds to specific X/Y coordinates (i.e. row/column number) and a colour class (from 1 to k). Take pairs of adjacent points along rows and columns of the grid sampling and record transitions among the colour classes (e.g. X number of 1-1 transition, Y number of 12 transitions, etc.). Accumulate all the transitions into a transition matrix, regardless of the direction of the transect. This will contain all the information of the pattern(s) and/or background for comparison. The two properties of the matrix to be compared are the off-diagonals (which estimate patch parameters) and the diagonals (which estimate patch areas). Summing the off-diagonals provide information on the frequency of colours adjacent to each other and inherently captures some components of the geometry of the pattern and its texture. Summing the diagonals and dividing by their total provides the relative area of each colour class, allowing for conventional colour analyses, as well as, allowing measures of colour diversity as an estimate of pattern complexity or visual contrast.

The complexity of a pattern is defined as the inverse Simpson's diversity calculated on the colour classes. Larger Simpson values means more even distributions. Using this complexity measure, permutation tests can compare relative frequencies for the different transition types. If the samples are distributed over the surface in a regular or random pattern, then the number of samples within each colour patch will be proportional to that patch's relative area. This allows statistical comparisons of the entire colour patterns, where differences between the diagonals and off-diagonals of two colour-pattern transition matrices, provides information on the degree of resemblance between the animal and background.

Second and higher order review

The studies described utilize the relationship between two or more neighbouring image pixels to determine the relationship between the coloration pattern and the background. These methods, with the exception of Endler's body pattern transections, rely heavily on digital images and the use of image statistics. It requires extra steps and more complicated mathematics to acquire data properly, but they have proven useful at comparing the spatial characteristics of the pattern to the background.

This makes finding edges easier and can determine the amount of change (e.g. colour, size, brightness) across a pattern. The strength in these methods compared to first order approximations is that complex patterns can be compared to the environment. We can consider the full pattern, rather than bits and sections in first order approximations. The downside from these methods is revealed when we realise that none of the described methods will determine if any given pattern will increase its camouflage efficacy. We can determine whether patterns possess similar qualities to the environment, even in regards to non-human vision, yet we cannot validate these models on their own.

While the first-order statistics are useful for comparing global characteristics, we can see the transition in the literature to utilising second and higher order methods. The need to reliably analyse local features in an image, beyond colour, has driven this change. With the rise of the digital age, image statistics have proven to be a useful tool to discover how visual systems interpret their environment in ranges of animals, beyond *Homo sapiens*.

For example, in the Fourier space, the magnitude, phase, and power spectral density (hereafter referred to as power spectra) are easily obtainable. Magnitude describes how much of a certain frequency component exists in the image; phase tells where the frequency lies in the image; and the power spectra describes the variation of intensity between neighbouring sets of pixels. Figure 1.4 illustrates three textures in their original state, phase randomized, and whitened. When the phase information is randomized, the detailed information for object recognition disappears and the images appear cloudy. Whitening occurs when the power is set to a fixed value (in this case 1) at all frequencies. The lack of intensity variation between pixels reduces the contrast significantly; however we can still interpret the textures from their original picture. This example demonstrates that cryptic coloration doesn't fully rely on the level of contrast between any two points, but information about spatial phase that represents localized features.

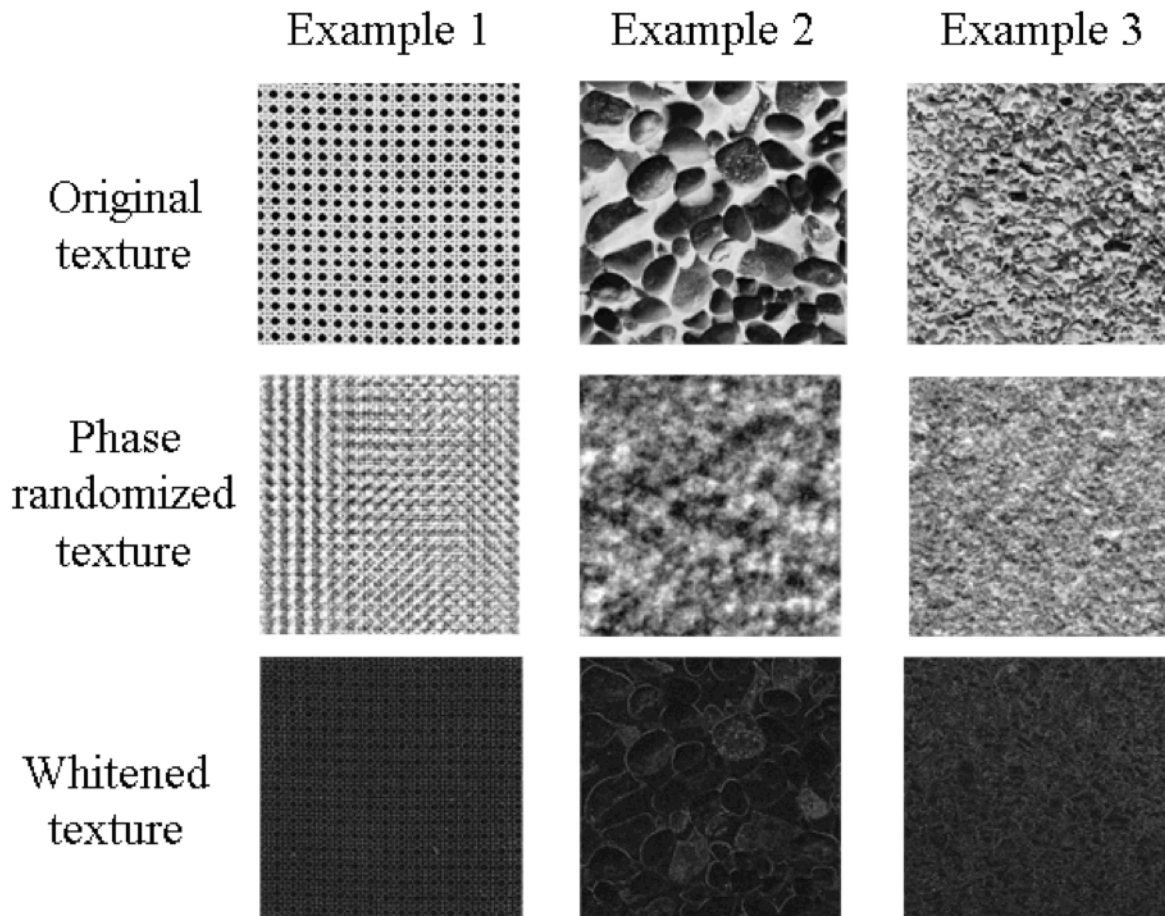


Figure 1.4: An example showing how the Fourier analysis may alter texture. The first row shows the original photos. The second row shows the photos when the phase has been randomized, leaving the image unrecognizable from the original. The 3rd row has the power fixed to 1, where the variation across each image is reduced, but we can still make out the original image.

1.4 Behavioural experiments and theoretical applications

Cryptic coloration does not always rely on image statistics to compare coloration patterns or to generate camouflage theories. The following two sections (behavioral analyses and theoretical applications) propose methods to validate or compare camouflage models with respect to the visual capabilities of predators and conspecifics.

Behavioural experiments

For all the methods previously described, there's no useful way of determining whether any metric for background matching or disruptive camouflage actually measures any degree of crypsis. The methods described measure the similarity between body pattern features

and its surroundings, but provide only assumptions for how cryptic a pattern should be in a given situation. Even more, how valid is any one method to another and how can we determine which measures adequately compare textures to their background? To fully understand how well our intuitions work in the natural world requires testing our methods and hypotheses behaviourally.

Behavioural experiments allow researchers to validate and test various camouflage hypotheses to live predators using real and artificial prey targets. Artificial targets allow researchers to manipulate pattern features and test how likely they are to survive to real predators, with the assumption that greater survival rate indicates better camouflage (i.e. the predator cannot as easily distinguish one pattern compared to other patterns). Targets are made using real or artificial textures whose colours and features are derived from theoretical approximations to the predator's visual capabilities and the survival rates of these patterns validate how well camouflage theories predict the responses.

Stevens and Cuthill (2006) created artificial moths to test two disruptive camouflage hypotheses against live bird predators. The relationship between background matching and disruptive camouflage strategies has been debated, with the discrepancy centered on whether disruptive patterns function separately to background matching patterns. Background matching patterns attempt to match a random sample of the background's colour and pattern to prevent recognition, however many patterns contain large, apparently conspicuous features. These large, high contrast markings were thought to disrupt prey recognition by breaking up the outline of the animal, creating false edges on the pattern. For disruptive camouflage to be most effective, it had been hypothesised that disruptive patterns required markings that cover the outline of the body and these markings worked best at high contrast. To test whether disruptive camouflage functions independently to background matching, the disruptive patterns must meet two conditions: 1) the distribution of colour patches matches that predicted by theory, and 2) these distributions reduce detectability of prey to predators. If disruptive patterns do not function separately to background matching patterns, then the survival rates between patterns overlapping the animal's outline (disruptive) and ones that do not (background matching) at high and low contrasts should show no difference in camouflage effectiveness.

The researchers conducted two separate experiments to examine the role of patterns covering the edge of a pattern and the level of contrast, respectively. Artificial moths were

created using digital images of oak trees (grey-scaled and thresholded at 50%). A random sample of the tree bark was used as the texture for the wings, making the wings unlike any lepidopteran pattern, but representative of natural scenes. Colour matches of treatments to natural bark were verified by spectrophotometry of stimuli and bark, followed by modelling of predicted photon catches of a typical passerine bird, the blue tit's (*Parus caeruleus*) single cone photoreceptors, with irradiance spectra from overcast skies in the study site. Dead mealworms were pinned onto the artificial moths made of coloured paper triangles. These were pinned onto oak trees in the Leigh Woods National Nature Reserve, North Somerset, UK and their survival checked at 2, 4, 6, and 24 hours.

In the first experiment, Cuthill and Stevens examined how edge markings affect camouflage. Five treatments of artificial moths were created. The first treatment used a pattern that covered portions of the moth's edge. The second condition was the same pattern as the first treatment, but the markings moved inward to keep the pattern from the edge. The third condition was a different oak bark sample to the first two and also did not touch the moth's edge. The fourth and fifth treatments were uniformly coloured black and brown controls. The results followed Stevens and Cuthill's hypothesis that the patterns covering portions of the edge (first treatment) survived more often than the two conditions with patterns not touching the edge, which all did better than the uniformly coloured, black and brown, prey conditions. The theory of disruptive colouration accurately predicted how the colouration patterns affected prey survival in the field.

In the second experiment, the role of contrast was examined on 6 artificial moth conditions. Like the first experiment, two moth conditions contained high-contrast patterns overlapping the edge and not overlapping the edge, with lower contrast variation of both patterns accounting for two other conditions [i.e. 1) high-contrast covering edge; 2) low-contrast covering edge; 3) high-contrast with no edge; 4) low-contrast with no edge]. The fifth and sixth conditions were uniformly coloured based on the mean colour of the high and low-contrast patterns, respectively (i.e. mean high contrast and mean low contrast). As predicted by the disruptive colouration theory, high-contrast patterns overlapping the edge showed the greatest survival rates followed by low-contrast patterns overlapping the edge. High-contrast patterns not covering the edge placed third followed by low-contrast patterns not covering edge with the uniformly coloured, mean high-contrast and mean low-contrast patterns with the worst survival rates, respectively. In both experiments, the presence of patterns overlapping the edge proved to be most important, with higher contrast surviving

better than low contrast, showing how field experiments validate our working hypotheses for how predator vision shapes the evolution of protective colouration.

Theoretical applications

Camouflage has predominantly been understood through the lens of human perception. Over the last century, technological developments enabled researchers to understand the eyes and visual systems of other animals, showing a wider range of capability and limitation outside the human scope. Even more, the physical medium affects how light is distributed and received by a perceptual system, especially in water. Combining these physical and visual components enables researchers to hypothesize about the functionality of body patterns and how human perception may not adequately characterize the functionality of body patterns and how they mask themselves from their non-human predators (Bennett et. al 1994).

One avenue of great interest in this research investigates how varying visual systems interpret colour. For each cone photoreceptor, researchers quantify how sensitive the receptors are to different wavelengths of light, enabling them to determine the smallest difference needed to discriminate two colours (i.e. the JND). Once researchers discover how colours are able to be discriminated in this colour space, questions can address how colours in the environment are perceived and to what degree the observer can reliably tell them apart.

While most camouflage uses a range of more earthy colours (e.g. green, black, white, brown), several habitats such as coral reefs possess numerous vibrant colours. As a result, certain animals within the reef systems have developed colourful and ornate body patterns, which seem anything, but useful camouflage. Marshall (2000) examined the functions of brightly coloured angelfish (*Pygoplites diacanthus*) and moon wrasse (*Thalassoma lunare*) using new non-subjective spectrophotometric measurements, quantifying the way the fishes appear in their aquatic habitat. For simplicity, we will refer to the work on the angelfish.

The blue and yellow colours of reef fish present a strong contrast to the human observer but few studies examine how the fish themselves perceives these colours. Marshall measured the visual acuity of the fish's retina, and the spatial (frequency) distribution of the animal's colours. These values were placed into a hypothetical dichromatic model visual system (an altered model based on Vorobyev and coworker's (1998) equations) to imitate the fish's sight and determine how the colours were observed (conspicuous or inconspicuous) at various distances against the open blue waters or coral substrates.

The visual acuity of the fish was obtained from previous research (Collin and Pettigrew 1989), giving a range of range of 7-27 cycles deg^{-1} for known reef fishes with most being close to 10 cycles deg^{-1} , based on the ganglion cell receptive fields. Body pattern spatial frequencies were obtained using resized photographs from Randall *et al* (1971).

Angelfish were discovered to have high contrast at a range of 1.33m from the altered Vorobyev model (Figure 1.5). The blue stands out against the coral environment yet blends with the water column very well. The yellow components strongly contrast the water column with a colour distance value of 1.27, standing out and being very apparent. The yellow does blend in with the coral environment up to 570 nm, and the dichromatic model generates a colour distance of only 0.04. The yellow stands out to the human observer against both backgrounds; however the fish eye cannot discriminate the yellow colours as well as human, allowing the yellow to blend. We're left with two colours that tend to contrast in both environments. Marshall believes this contrast is due to one of two reasons: firstly, the colours stand out to signal territory to other fish, or secondly, that the colours act as a disruptive camouflage. Angelfish live near the coral areas, and swim to coral branches when startled, enabling the yellow and blues to blend with the yellow coral and blue water column, breaking up the fish's outline.

Marshall's technique is unique in that it utilizes current physiological information to generate a hypothesis for how coloration masks brightly coloured animals. However, the information should be tested in natural or laboratory conditions against predators to determine the effectiveness of the hypothesis

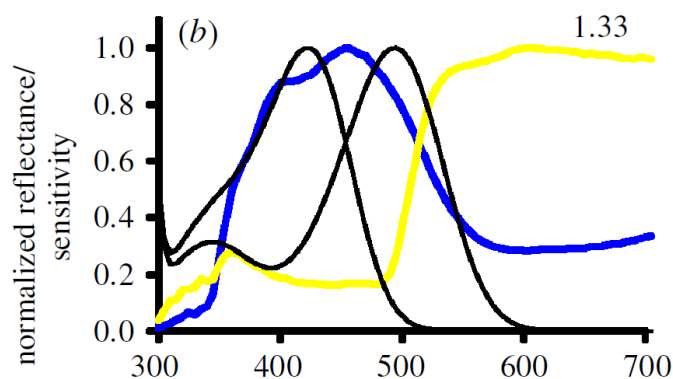


Figure 1.5: Reflectance of yellow and blue in *P. diacanthus*. Both colours normalized to the peak of blue. The two colours are complementary, that is, where one reflects strongly, the other one does not and vice versa. In the top right-hand corner the black 1.33 refers to the colour distance between yellow and blue, a measure of the contrast of the two colours, for the

dichromatic system described in ½ 2. The thin black curves represent the spectral sensitivity of the visual pigments of this dichromatic system with absorbance (λ_{max}) peaks at 424 nm and 494 nm (Marshall 2000).

1.5 Conclusion

Here we have reviewed the methods used to quantitatively compare camouflage patterns to their backgrounds. While the first-order statistics are useful for comparing global characteristics, we can see the transition in the literature utilising second and higher order methods. The need to reliably analyse local features in an image, beyond colour, has driven this change. With the rise of the digital age, image statistics have proven to be a useful tool to discover how visual systems interpret their environment in a range of animals, beyond *Homo sapiens*.

Effective camouflage utilizes more than colour-matching in a pattern. Schaefer and Stobbe (2006) compared the survival performance between disruptive and cryptic coloration on artificial moths, resembling peppered moths, against white birch trunks (matching background) and mossy tree trunks (mismatching background). Disruptive coloration included colors that matched the background as well as patterns with conspicuous colors such as blue and pink. Their data showed disruptive markings with lower predation rates than background matching due to poor background matching performance against mismatching backgrounds. The mismatching colors fared just as well, if not better, than background matching moths, suggesting disruptive coloration is indeed independent from background matching. The lower predation rates for disruptive patterns on mismatching backgrounds suggests that disruptive coloration enables the wearer to exploit a larger range of habitats compared to background matching patterns.

From these examples, we can see how utilising second and higher order statistics can prove to be a powerful tool in examining complexities between the pattern and the background. The issue with these methods lies in our inability to determine whether any of these methods can reliably determine whether a pattern will camouflage an animal.

The digital age has introduced new, powerful algorithms and methods to tease out the subtle features used in image registration, segmentation, and identification. This is evident with the complexity of analyses discussed in the 2nd and higher order statistics section. However, newer, powerful techniques have been developed that have yet to be utilized in the literature. These have been implemented in the private sector for Google, Facebook, and

other multimedia companies to provide faster, more accurate services (Mikolov et al 2013; Piantadosi 2014). These methods have large potential in helping discover what features between the pattern and the environment are necessary to prevent detection.

The focus of this thesis rests on the implementation of a subset of these new techniques to tease out how the common cuttlefish (*Sepia officinalis*) orchestrates its patterns to camouflage to a wide range of environments and to discover what, if any language-like properties exist during intraspecific communication. Chapters 2 and 3 focus on how cuttlefish orchestrate the various spots, stripes, and other pattern markings to camouflage to diverse background types, while Chapter 4 investigates the information content present in cuttlefish communication. During our investigation into cuttlefish communication, we discovered 2 previously undocumented patterns used by cuttlefish. My fifth and final chapter describes these patterns and the contexts they were displayed.

2. Camouflage strategies of the common cuttlefish (*Sepia officinalis*)

2.1 Introduction

The natural world has a beautiful complexity; its diverse colours, patterns, and textures challenge camouflage patterns to remain effective against multiple backgrounds. Effective camouflage is essential to an individual's survival, where the appearance and behaviour affects how the predators learn to perceive and distinguish their prey (Skelhorn and Rowe 2016). Some species move to more concealing substrates to better aid their camouflage (Kang et al. 2013; Tyrie et al. 2015), and it seems that some species can assess the relative quality of backgrounds for concealment. For example, the ground-nesting Japanese quail (*Coturnix japonica*) lays its eggs on substrates depending on the degree of maculation (i.e. spots) on the eggs. Eggs with heavy maculation are laid on substrates matching the maculation, utilising disruptive camouflage, whereas eggs with light maculation are laid on substrates matching the egg colour, utilising background matching (Lovell et al., 2013). Camouflage research examines the relationship between the prey and the substrate, teasing out the visual cues preventing predator detection, and thereby providing a glimpse into the perceptual capabilities of both the predator and prey. Research primarily focuses on how prey patterns prevent initial detection, and more recently how quickly predators detect different forms of camouflage over successive encounters (Stevens et al. 2012; Troscianko et al. 2013). What is not as well understood is how prey integrates their behaviour and coloration pattern(s) to disrupt predator learning.

Animals optimise their foraging to receive the highest nutritional value with the lowest effort. So how do predators optimise their hunting strategies to polymorphic prey or multiple prey species? It is commonly assumed that animals operate under perceptual and cognitive constraints: they have limited capacities for attention and problem solving (Guilford & Dawkins, 1987). It follows then that predators should focus on the most abundant prey-types, minimising the effects of these constraints (Dawkins, 1971; Bond, 1983; Greenwood, 1985; Dukas and Ellner, 1993; Dukas and Kamil, 2001; Dukas, 2002). Given such limitations, one longstanding theory has been that predators increase their rate of prey detection through search images. Tinbergen (1960) found that when great tits (*Parus major*) prey on various species of insect larvae, the rarer morphs in the population were eaten

disproportionately less frequently than common morphs. This is consistent with the idea that the predator's successive encounters with the most common prey-types trains its perceptual mechanisms to detect them, at the cost of the ability to detect rarer morphs.

Published literature on facilitation suggests that repeated exposure to a stimulus increases detection rates with a prey-type, but whether these successive encounters interfere with the performance on other prey-types remains unclear (Posner & Snyder, 1975; Guildford & Dawkins 1987; Plaisted & Mackintosh 1995; Plaisted 1997). Putting these controversies aside, the literature does demonstrate that the degree of crypsis of the prey-type influences the learning rate of predators (Punzalan *et al*, 2005). When animals are trained with conspicuous targets, they fail to detect more cryptic alternatives, however if the animal is trained on a cryptic target, no interference responses occur (Blough, 1989, 1992; Bond & Riley, 1991; Lamb, 1988; Reid & Shettleworth, 1992).

In the camouflage literature, recent advances have sought to reinvigorate our categorisation and definition of camouflage types (Stevens & Merilaita, 2009; Stevens & Merilaita 2011). Crypsis, the form of camouflage meant to prevent detection by blending into the background, is subdivided into background matching and disruptive camouflage. Background matching patterns, as the name implies, match the colour and pattern to seamlessly blend with the background. Disruptive camouflage uses high contrast markings to break up the outline of the animal. Researchers have investigated the differences between these two strategies, attempting to determine whether any *functional* differences lie between them. There's no question as to whether either function well as visual camouflage, but rather whether some components of disruptive coloration enhance background matching, thereby blurring the traditional distinction between the two strategies (Hanlon et al., 2009). While camouflage studies primarily examine the visual cues used to prevent initial detection, recent efforts have compared detection rates on different types of camouflage over successive encounters. Troscianko and coworkers (2013) tested detection rates of multiple camouflage types (distractive, disruptive, and background matching stimuli of varying contrast levels) on human observers. Distractive markings - such as eyespots - while rarely tested, differ from the two primary forms of crypsis, by using conspicuous markings to draw the predator's attention away from the outline of the animal. They discovered that disruptive camouflage offered the best camouflage at initial detection, while distractive marks fared the worst. More interestingly, they discovered that high contrast markings were learnt more quickly compared

to the background matching stimuli. In other words, while disruptive camouflage may be the most difficult to detect initially, it is the easiest to learn.

In all the studies discussed, prey have fixed patterning that rarely changes over the course of their lifetime. Some animal groups/taxa, however, possess the ability to alter both the colour and visual texture of their patterns. These include flatfish (Pleuronectiformes), and chameleons (Chamaeleonidae), but the masters are the coleoid cephalopods (squid, octopus, and cuttlefish) (Hanlon & Messenger 1996). These molluscs can change their appearance in under a second, providing a unique opportunity to examine camouflage in ‘real time’. With their rapid colour changing capabilities and an acute visual system, similar to most vertebrates, cephalopods are increasingly popular models in camouflage studies.

The speed and flexibility of cephalopod pattern change comes from the thousands of pigment-filled sacs distributed across the skin, known as chromatophores. The chromatophores coordinate their expression through contraction and expansion to create the myriad spots, stripes, squares, and other features of the pattern, referred to as chromatic components. Through extensive observation, researchers have identified 34 chromatic components, which cuttlefish combine to create 12-14 body patterns used to signal to conspecifics and camouflage (Hanlon and Messenger 1988; Crooke et al. 2002). Patterns are not as well defined as one would presume. Patterns are defined by a few ‘primary’ components, allowing a wide range of potential ‘subcomponent’ expression. In other words, a pattern is defined by the expression of a few primary components, but may encompass a wider range of subcomponents. While research has focused on what aspects of the environment elicits the expression of the primary components, the degree of flexibility of these subcomponents and what elicits their response is not well understood (Barbosa et al. 2008; Chiao and Hanlon 2001; Chiao et al. 2015).

In our previous research, we noticed that cuttlefish (*Sepia officinalis*) frequently change their pattern, on natural backgrounds such as gravel, and also on more controlled and simpler patterns such as checkerboards. On natural substrates this might be because the animals respond to local variation in the background, but checkerboard backgrounds lack such variability, and the cuttlefish continued to change their patterns throughout each experiment.

The observation that cuttlefish change their appearance on a fixed background suggests two strategies that cuttlefish might use: 1.) To change the pattern over a short

timeframe, but eventually settle on the optimal pattern, or 2.) Cuttlefish continually change their appearance. In the latter case one can also ask how the patterns change in appearance and over time. For example do they switch at regular intervals between two patterns, or is the behaviour more stochastic, and if so what are the statistical characteristics of the changes?

Cuttlefish are sensitive to the contrast and size of objects surrounding them. For example, the expression of the disruptive pattern is highly correlated to objects similar in size to their white square (Chiao & Hanlon, 2001). More importantly, we wanted to understand how the variation in the environment influenced both the number and frequency of changes of patterns in the environment. We expected that natural environments, possessing greater variation, would elicit higher numbers of patterns with more frequent changes. Furthermore, we wondered how these pattern variations compared to their background.

To record pattern changes we employed a 2-D log-Gabor filter, which is a linear filter used for edge detection and texture feature extraction (Baddeley & Tatler, 2006). We trained a logistic regression model on random samples of the background, and tested whether a given cuttlefish pattern would be classified as belonging to its respective background or as a cuttlefish. This allowed us to quantify how often a given cuttlefish pattern was misclassified as the background. Higher rates of cuttlefish patterns being misclassified as the background would demonstrate a greater visual similarity between the pattern and its respective background, suggesting better camouflage.

2.2 Methods

Subjects

Cuttlefish eggs were collected from the south coast of England and were kept at the SeaLife centre, Brighton, UK in which they were hatched. Cuttlefish hatchlings ($n=10$) were fed ad libitum on mysids and naupliids and were kept under a 12-hour light-dark cycle. Subjects were approximately 4 months old at the start of the experiment with an average mantle length of 36.87mm (std. dev. = 2.75mm).

Stimuli

We produced three artificial and three natural backgrounds to compare the background variation with cuttlefish camouflage patterning (Figure 2.1). The artificial backgrounds consisted of black and white checkerboards (3, 8, and 12mm sized checkers).

These test stimuli have long standing history of producing the range of uniform, mottle, and disruptive body patterns. The natural backgrounds composed of sand, pebbles, and stone, also known to produce the uniform, mottle, and disruptive patterns, respectively (Langridge 2006). The variation in size, contrast, and depth provide larger statistical differences compared to the artificial checkerboards (fixed to a specific size and contrast).

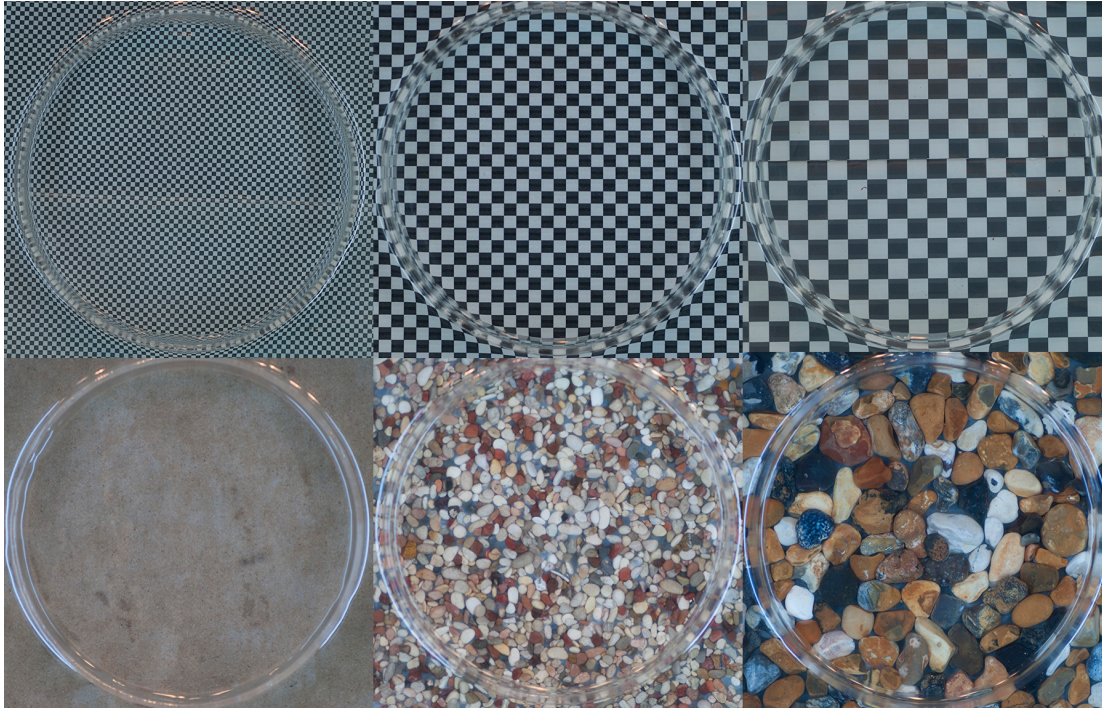


Figure 2.1: Top row: The 3 artificial checkerboard backgrounds (3mm, 8mm, and 12mm) from left to right. Bottom row: The 3 natural backgrounds (Sand, Pebble, and Stone) from left to right.

Experimental setup and procedure

Ten cuttlefish were tested individually inside a circular arena made of Perspex with a diameter of 22cm and a height of 10 cm, which was placed inside an experimental tank that minimised disturbance (Kelman et al., 2007). Images were acquired with a Nikon D5000 digital camera. Backgrounds for each condition were placed inside the tank with the Perspex ring sitting on top.

Animals were placed inside the arena with images taken every 5 seconds for 30 minutes with a total of 360 images per run, for a total of 21,600 images obtained. Cuttlefish were monitored for signs of undue stress during the trial periods and only handled once per day.

Frequency and number of patterns used

A change in pattern was defined as the addition/subtraction of a component or the level of its expression on the cuttlefish, based on the components described by Hanlon and Messenger (1988).

The total number of unique patterns was calculated for each run and averaged for each condition. A MANOVA was performed to compare the averages between each condition.

To determine the frequency of pattern changes, we used images recorded every 10 seconds. A cuttlefish image was compared to the previous image in the run, accounting for the components expressed. The number of changes was grouped into 5-minute bins to obtain the probability a cuttlefish pattern would change in the 5-minute timespan. A MANOVA was performed to compare the frequencies between each condition.

Texture comparisons

The cameras used had not been calibrated, therefore no colour information can be used, only the texture information. A 2-D log-Gabor filter which is a linear filter used for edge detection and texture feature extraction (Gabor 1946), was used to quantify and compare the textures of backgrounds and cuttlefish separately (for the texture match). The MATLAB function was provided by David Gibson, Department of Electrical & Electronic Engineering, University of Bristol.

First, the images were hand-labelled in Paintshop Pro using the Paint Brush tool set at 5 pixels width and 100 Hardness to define region boundaries. The reason to use a relatively thick tool was to ensure that only regions that could be defined unambiguously were labelled; exact boundaries were sometimes difficult to identify (or draw precisely) and so these border regions, which might contain elements of cuttlefish and background, were excluded (Figure 2.2). Then Flood Fill tool was used to fill the same colours for cuttlefish (R: 0, G: 128, B: 0), background (R: 128, G: 0, B: 0), boundaries (R: 224, G: 224, B: 192) and any region of the photograph not falling in these categories (R: 0, G: 0, B: 0), for all images to define regions. These regions were then false-coloured using the PASCAL colour-coding scheme, a universal standard for region labelling for ground-truthing in computer-based image segmentation (Everingham et al. 2014). The segmentation maps (e.g. Figure 2.2, right panel) were saved as PNG files (Noninterlaced and Optimized palette) and used subsequently to identify, extract, and rotate (cuttlefish standardised with the caudal-up and rostral-down) the

cuttlefish and background regions automatically from the corresponding photograph (e.g. Figure 2.2, left panel), using a MATLAB program.

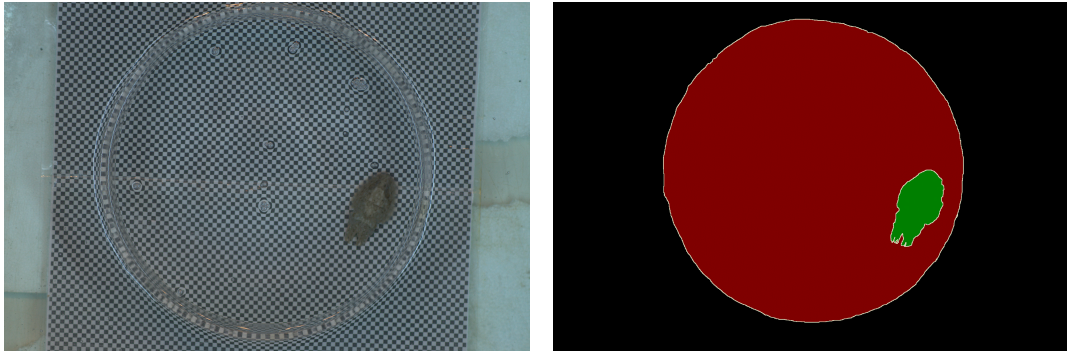


Figure 2.2: Image labelling according to the PASCAL standard. Left: original photograph. Right: hand-labelled segmentation map of the regions: background (red), cuttlefish (green), boundary (white) and non-target areas to be ignored (black).

The background and cuttlefish regions of each image were then convolved with the 2D log-Gabor filter bank. Data representing the texture of each image was quantified by convolving the luminance plane with a bank of 24 filters: 6 orientations by 4 spatial scales, to describe the textures in 24-D space. The mean amplitude of each filter's output for each image section was then calculated. The image sections analysed were, for each photograph, the cuttlefish itself plus 5 sections of background with the same size of the cuttlefish (157 x 79 pixels) randomly sampled from within the background region in that photograph (using Matlab's random number generator to generate coordinates). Thus we could compare the texture of the cuttlefish with same-sized samples of background allowing comparisons within and between cuttlefish and backgrounds, with each cuttlefish and each background sample being defined by a point in 24D space.

To compare the camouflage efficacy of the cuttlefish patterns, we took out the cuttlefish patterns and trained a logistic regression model on the background data only. We then tested whether the cuttlefish patterns were predicted as the background or as a cuttlefish. In other words, we wanted to see if the cuttlefish patterns were distinguishable from the background samples based on their visual similarity. High rates of misclassification (i.e. the model incorrectly categorises a cuttlefish pattern as being a background sample), suggests a better pattern match. We used a chi-square test (SPSS version 23) to compare significant differences between classification rates.

2.3 Results

Average patterns used

We found significant differences for the average number of patterns used between each condition, however the results do not confirm our hypotheses that natural backgrounds would elicit more pattern changes than checkerboards. The average number of patterns did not differ based on the natural vs. artificial conditions we placed them, but the size of the objects on the substrate did have an effect (Figure 2.3). The 3mm checkerboard averaged 8.1 patterns; sand averaged 8.6 patterns; the 8mm averaged 11.1 patterns; pebble condition averaged 13 patterns; 12 mm checkerboard averaged 13.9 patterns; and stone averaged 24.4 patterns.

The stone condition produced the largest average number of patterns, and was significantly different from all other conditions (all p -values <0.001). The pebble and 12mm checkerboard both differed from the 3mm and sand conditions ($p<0.05$), but not the 8mm checkerboard conditions. The 8mm checkerboard condition only differed from the stone condition, while the sand and 3mm checkerboard conditions differed from everything, but each other and 8mm ($p<0.05$).

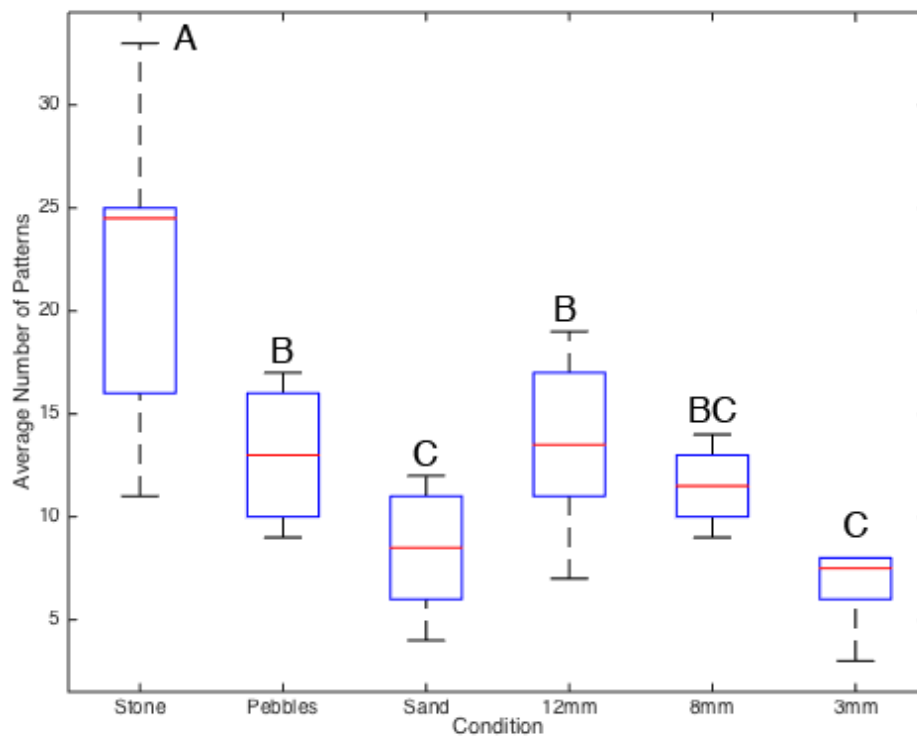


Figure 2.3: The average number ($n=10$) of patterns (y-axis) for each condition (x-axis) showing that the average number of patterns increases with respect to the size of objects on the substrate. The box and whisker shows the range of patterns expressed by individuals on each background. The blue box represents 50% of the data, with the red line marking the median number of patterns expressed. Within the box, 25% of the patterns were expressed above the red line and the other 25% below the red line. The other 50% of patterns expressed lie along the dashed lines (i.e. the whiskers). Letters represent which conditions were significant from each other.

Frequency of patterns used

We determined that the cuttlefish continually change their patterns over the course of the 30-minute trials (Figure 2.4). The frequency reduces over time from a 60% chance of changing in the first 5 minutes, gradually decreasing to a 40% probability of changing in the 25-30 minute range.

We found a significant difference in pattern changing frequencies between artificial and natural conditions, however the trend moved opposite to our hypothesis. Artificial patterns changed more frequently compared to natural conditions (Figure 2.5). The 3mm and 8mm checkerboard conditions differed from all the natural conditions, with the exception of the pebble condition ($p<0.05$). The 12mm checkerboard differed from all the natural conditions ($p<0.05$).

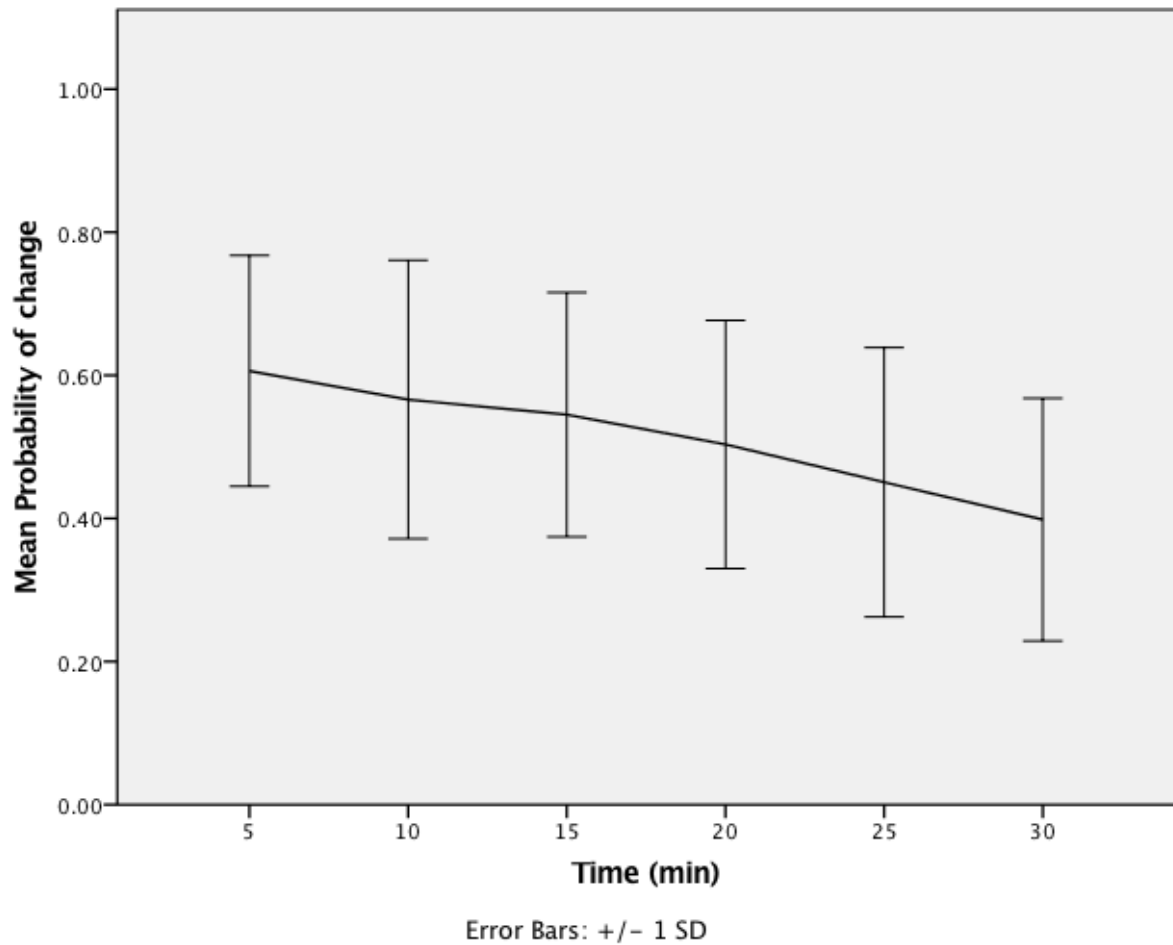


Figure 2.4: The mean probability a cuttlefish ($n=10$) changing its pattern (y-axis) over the course of 5-minute intervals (x-axis) for all backgrounds. The mean probability decreases over time from 60% to 40%, over the 30-minute interval, and the error bars demonstrate that for every time interval, no matter the background, the cuttlefish continually alter their patterns. Once we determined cuttlefish continually change, regardless of background, further analyses investigated how backgrounds influenced the probability of change (See Fig. 2.5).

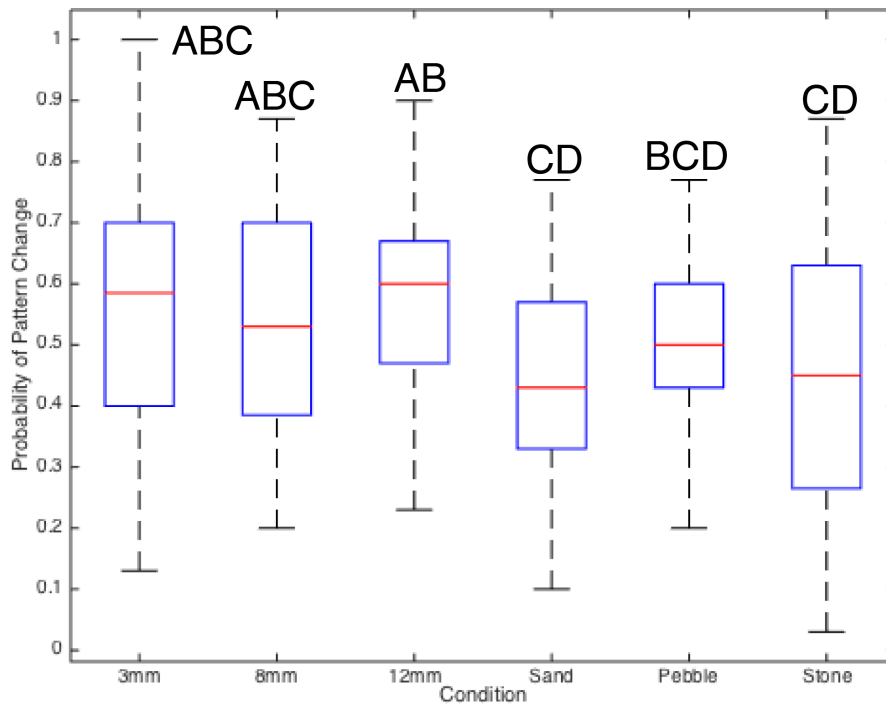


Figure 2.5: The probability of a cuttlefish changing its pattern (y-axis) for each condition (x-axis). Artificial backgrounds produced more frequent pattern changes compared to the natural conditions, with the exception of the pebble background. The box and whisker shows the range of patterns expressed by individuals on each background. The blue box represents 50% of the data, with the red line marking the median number of patterns expressed. Within the box, 25% of the patterns were expressed above the red line and the other 25% below the red line. The other 50% of patterns expressed lie along the dashed lines (i.e. the whiskers). Letters represent which conditions were significant from each other.

Texture comparison

Our χ^2 test found a significant effect ($p < 0.001$) between the conditions. We compared the columns to determine how each condition differed between their background vs cuttlefish classification frequencies. Every condition resulted in a significant classification difference (Figure 2.6). The patterns displayed on artificial backgrounds were reliably classified as being a cuttlefish pattern, while patterns displayed on natural backgrounds differed. The patterns displayed on sand and pebble backgrounds were classified as being part of the background, suggesting good background matching, while the patterns displayed on the stone background were classified as cuttlefish. While all the patterns displayed on artificial conditions were reliably classified as a cuttlefish, as the size of the checkers increased, so did the misclassification ratio. With the patterns displayed on natural conditions, pebble

condition patterns were categorised as the background more often to sand condition patterns, while stone condition patterns had more equal rates of classification, but significantly more cuttlefish categorisations.

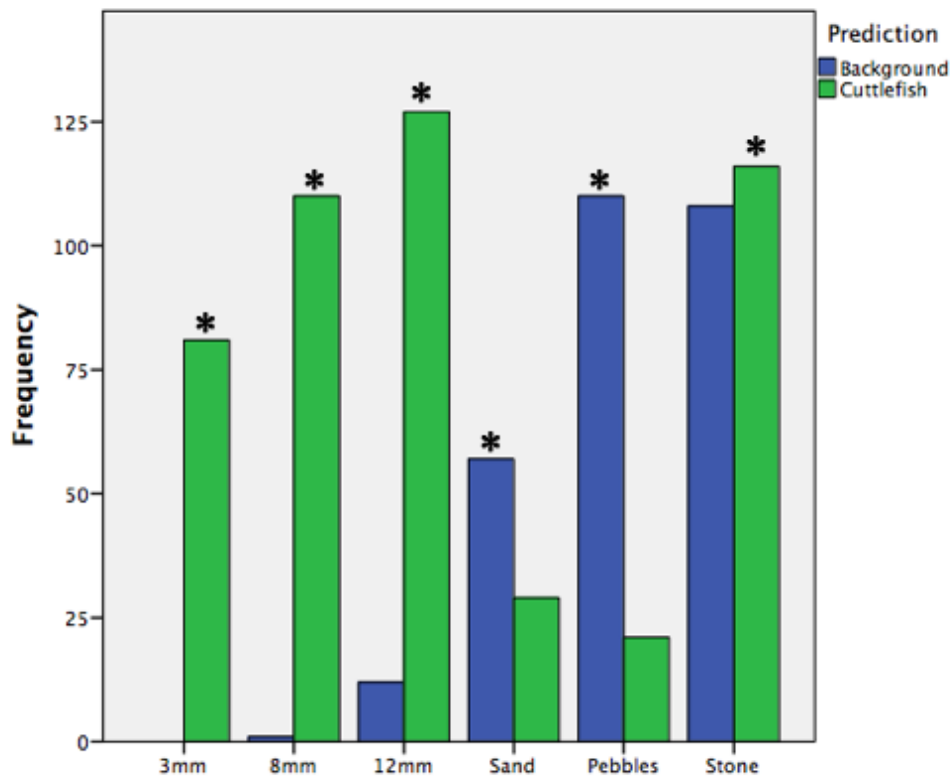


Figure 2.6: A bar graph displaying the frequency of logistic regression classification for a given pattern as being a cuttlefish (green bars) or background (blue bars) for each condition. Significant differences are indicated at the 0.05 level.

2.4 Discussion

Our experiments reveal that cuttlefish continually alter their camouflage patterns over the course of the 30-minute trial period (Figure 2.4), however the frequency of pattern changes and the number of patterns employed depend on the characteristics of the substrate.

For the number of patterns used on a given background, we predicted that natural backgrounds would elicit more pattern variations compared to artificial checkerboards. The logic behind this supposed that the variation in natural backgrounds (varying in size, colour, depth, etc.), especially in the pebble and stone conditions, would produce patterns utilising larger numbers of components compared to artificial checkerboards (varying only in size with

a fixed contrast). We discovered that the range of patterns depended on the size of objects on the substrate (Figure 2.3). Conditions with larger objects, namely the 12mm checkerboard and stone conditions, produced the largest amount of pattern variation, reducing the degree of variation to the smallest sized objects on the 3mm checkerboard and sand conditions. Cuttlefish use this same principle of pattern selection in the broadest categories of camouflage patterns (e.g. uniform, mottle, and disruptive), where the expression of the white square component depends on the size of the objects on the substrate (Chiao & Hanlon, 2001). This makes sense, as a larger number of components are expressed in disruptive patterning compared to mottle and uniform patterns. This tactic may benefit the cuttlefish through the use of a more varied disruptive repertoire, as disruptive patterning may be the most difficult to initially detect by predators, but is the fastest to learn (Troschianko et al., 2013).

Using the same logic with pattern variation, we assumed the variation in natural backgrounds would increase the frequency of pattern changes compared to artificial checkerboards. Interestingly, we found the opposite – artificial backgrounds elicited more frequent changes compared to the natural backgrounds (Figure 2.5). The lack of variation on the artificial conditions may influence cuttlefish to continually alter their appearance to disrupt a predator's pattern search formation. Alternatively, the natural backgrounds provided a textured substrate cuttlefish are used to. The laminated checkerboard sheets possess a smooth, glossy texture cuttlefish might find aversive, increasing their rate of movement and subsequently their rate of pattern changes (Zylinski et al., 2009b).

Considering that cuttlefish continually alter their patterns, we wondered how often they used patterns optimising their camouflage, and to what degree the pattern textures compared to the substrate. The logistic regression classified a pattern as either being part of the background (background) or not part of the background (i.e. a cuttlefish). Classification depended on the degree of visual similarity to the background. We trained the model using background-data only; allowing us to determine how similar a given pattern was to the background it was displayed. Unsurprisingly, cuttlefish poorly matched the artificial checkerboards (Figure 2.6). The small rate of patterns being classified as the background for the 8mm and 12mm can be attributed to the visual similarity of certain pattern components with the size of the checkers. For example, the large checks on the 12mm would possess a similarity to the white square on the disruptive pattern that cuttlefish usually display, while the smaller 8mm checkers might have some visual similarity to the mottling pattern. The

uniform pattern usually displayed on sand substrates lacked any similarity to the 3mm background, as measured by our Gabor filters.

Our primary interest is, however, in the animals' camouflage capabilities on natural backgrounds, where we find stronger visual similarity between the pattern and substrate. Patterns displayed on the pebble condition showed the strongest visual similarity (Figure 2.6), where patterns were misclassified as being part of the background (i.e. showed better background matching) most often, followed by patterns displayed on the sand condition. Interestingly, the patterns displayed on the stone condition were classified as being a cuttlefish more often than background (but not near as often as any of the artificial backgrounds). Cuttlefish displayed a greater proportion of disruptive patterning on the stone background, suggesting the disruptive patterns do not possess strong background matching characteristics. This does not imply that the disruptive patterns do not act as adequate camouflage. The Gabor filter comparisons we employed examine the visual similarity between the pattern and background, and does not account for how well the disruptive patterns break up the outline of the animal. This discontinuity between the natural patterns supports the notion that background matching and disruptive patterning operate as distinct camouflage tactics.

Whether our findings can be characterised as a search image formation avoidance strategy is debatable. The literature on search images does not account for rapid camouflage organisms, much less that the less used patterns benefit from image formation on the most commonly used patterns. What we can suggest is that cuttlefish continually alter their camouflage and that the number of patterns used [and the rate of change] depends on the size of the objects in the environment. The patterns displayed show a good match on non-homogenous backgrounds (e.g. sand), with the best matches on pebble-like backgrounds.

3. Component coordination in the camouflage patterns of the common cuttlefish (*Sepia officinalis*)

3.1 Introduction

Cephalopods are the unrivaled masters of camouflage in the animal kingdom, capable of changing the colour and texture of their entire body in under a second. This ability comes from the use of thousands of pigment-filled sacs distributed across the body, known as chromatophores. Cephalopod chromatophores differ fundamentally from those of other animals, such as fish and amphibians, in that rather than being controlled hormonally, they are a neuromuscular organ, being activated by motoneurons dependent on visual input from the environment (Messenger 2001). These chromatophores coordinate to create the spots, stripes, squares, and bars across the body, known as chromatic components. Thirty-four chromatic components have been described in the European cuttlefish (*Sepia officinalis*), which cuttlefish use in mixed combinations to create the various body patterns used in camouflage and signaling to conspecifics (Hanlon and Messenger 1988). With a theoretical possibility of 2^{34} potential patterns, it is an interesting question to ask how component expression is coordinated during natural camouflage behavior, and what is the full range of patterns that the cuttlefish can express. This suggests one of two hypotheses: either that the cuttlefish is capable of expressing the chromatic components independently of one another, or that their expression is constrained to a comparatively small number of discrete states (Crooke et al., 2002; Hanlon et al., 2009). A useful comparison is with human facial expressions, where the coordinated contraction/expansion of facial muscles may be continuous, but facial gestures can be categorized into discrete states such as smiles or frowns (Harris et al., 2012). We investigated component expression coordination by implementing a Gaussian Mixture Model to describe the range of components expressed, on a range of body patterns, to visualise how the components coordinate their expression and how cuttlefish transition between patterns in this body pattern space.

Coordinated Component Expression

Based on extensive observations in the field and aquaria, Hanlon and Messenger (1988) described 13 body patterns displayed by European cuttlefish, five of which are used

for camouflage. The 13 body patterns are divided into 6 chronic patterns (expressed for hours at a time) and 7 acute patterns (lasting for seconds or minutes) (Hanlon and Messenger 1988). Five of the six chronic patterns correspond to camouflage patterns (e.g. uniform, stipple, light mottle, dark mottle, and disruptive). Subsequently, Hanlon (2007) simplified the classification of these five patterns in favour of the so-called uniform-mottle-disruptive (UMD) scheme, which – he argued – could be applied to other animal camouflage patterns. Within this scheme, the uniform and stipple patterns are grouped together and the two mottle categories combined. Uniform patterns have little-to-no variation across the pattern, possessing small spots across the body for the stipple variation. These are typically used by cuttlefish to blend into sandy substrates. Mottle patterns have small spots distributed across the body, excellent for camouflaging to small pebbles and stones with more variation in the environment. While uniform and mottle appear to camouflage through background matching, disruptive camouflage includes high contrast markings, such as stripes and spots, which break up the outline of the animal.

These main body patterns are, however, variable. Each pattern is identified by the expression of a few key components, but other components can vary. We can understand the five camouflage patterns as prototypes, of variable phenotypes (Hanlon et al., 1999). The full repertoire of possible subcategories and their constituent components is not well understood. Hanlon and Messenger's scheme contributed greatly to camouflage and cephalopod research, yet discovering the full range of patterns requires a more detailed and quantitative approach. Since the initial body pattern classification, two methods have been proposed to account for the full range of patterns. These methods are respectively based on granularity analyses and Bayesian generative models.

Barbosa and coworkers (2008) implemented a granularity analysis to discriminate patterns based on the spatial scale of objects on the pattern. Using isotropic (i.e. circularly symmetric) filters which measure image power in six spatial frequency bands. For example, disruptive patterns with large, high contrast patterns contain relatively more low spatial frequency power than mottle patterns. In summary each pattern in the UMD classification possesses higher responses in certain spatial frequency bands. The difficulty here is that the unique signatures depend upon the presence of a few components, whose spatial scale determines the pattern in question. As Hanlon and coworkers (2009) state, a major challenge in defining cuttlefish patterns comes from shared components. For example, a cuttlefish may

express a uniform pattern with the white square component or a mottled pattern with the white square. As of yet, no method has been able to distinguish these mixed cases.

Crooke and coworkers (2002) attempted to automate the classification of the total number of patterns produced by cuttlefish through the use of a Bayesian generative model. Cuttlefish were photographed on a range of backgrounds and the components on each image identified as being present or absent. The data was put through AutoClass, a program known for finding clusters of stars in infrared and patterns in DNA/protein sequencing (Cheeseman & Stutz 1996). By examining the absence/presence of components on a range of patterns, AutoClass infers the number of potential patterns, based on the posterior probability of component expression. They found the cuttlefish utilise a range of 12-14 chronic and acute patterns, which was consistent with the conclusions of Hanlon and Messenger (1988). The range of patterns revealed by this method demonstrate a nice range of camouflage and signaling patterns used by cuttlefish, however five of the twelve patterns possess zebra stripes, commonly attributed to a single pattern type, referred to as the zebra display. In addition, the range of displays failed to produce several well-known patterns, including one of the most common camouflage patterns, known as the strong disruptive display. This suggests our knowledge of the full range of patterns and subsequent subcategories is not complete, requiring further investigation.

While the full potential of camouflage patterns is not fully known, researchers have extensively examined how particular aspects of the environment elicit certain components. For example, the white square, a prominent component associated with disruptive patterning, is expressed when objects in the environment are similar in size to the white square of the cuttlefish (Chiao and Hanlon 2001). Mathger and coworkers (2006) developed a handscoring method, a popular method for analysing cuttlefish patterns, where each of the chromatic components is scored on a scale from 0 (not expressed) to 3 (the component is fully expressed) (See methods section for further details). Kelman and others (2008) elaborated this method by using principal components analysis (PCA) to characterize the correlational values for the components associated with these orthogonal factors. The method has been used in a range of studies of cuttlefish camouflage (Barbosa et al. 2008; Zylinski et al. 2009b) and it turns out that the first one or two PC's tend to correspond to a set of components belonging to a recognized body patterns, such as disruptive or mottle, but higher order PC's usually do not, clearly pointing to (if not proving) independent modes of control. Using this method, we cannot determine the range of disruptive, mottle, or uniform patterns displayed

on a given background, but only their broadest category in the UMD classification scheme. For example, while the components may correspond to the mottle pattern, the scheme is unable to determine the intensity or level of contrast that defines the light mottle or dark mottle categories for cuttlefish. We wondered whether we could use the handscoring method, in conjunction with a gaussian mixture model (GMM), to cluster the range of patterns and visualise how cuttlefish coordinate their components to create patterns and transition between patterns.

GMM's are powerful tools to identify discrete categories within a dataset using undefined parameters. In other words, we can use the GMM to find discrete categories (i.e. patterns) of cuttlefish patterning, based on the visual similarity of patterns, without training the model to predefined concepts such as the UMD classification (Hanlon 2007). PCA is a dimension reduction method that finds the correlational values for each component with an independent variable of choice (e.g. background contrast). Our dataset quantifies the expression 23 different components, creating 23 dimensions. PCA allows us to reduce the dimensionality based on how likely the a component varies the dataset. Applying the GMM on to the data parametrized by the PC values allows us to find structure within the data – for example clusters or categories. By using the handscoring method over a linear filter for edge detection, we can classify body patterns based on the expression of specific components. We can plot the data and observe how distinct each pattern (i.e. cluster) is from other patterns by observing the distance between the pattern groups. If component expression is not independent, we expect to see gaps between the clusters, indicating that component expression is highly correlated for a given body pattern. If we fail to see this distinction, then we can suggest that components are more independent than previously believed. We used this clustering method over others, such as k-means, for two main reasons. First, GMMs adjust the 'distance' to each cluster – and thus cluster membership – based on the covariance of points within the cluster. Second, GMMs allow determination of the optimal number of clusters based on the Bayesian Information Criterion. Specifically, if there are distinct body patterns (Hanlon & Messenger 1988) we can predict that the PCA space will possess distinct boundaries between clusters, indicating discrete categories within the data. We follow the UMD classification and predict at least 3 distinct categories.

Cuttlefish pattern transitions

Cuttlefish body patterns are not fully autonomic responses to the environment. Cuttlefish, like all cephalopods, possess higher cognitive capabilities, demonstrating

impressive insight into the world around them. Langridge and coworkers (2007) showed that cuttlefish are able to assess the threat-level of potential predators, displaying predator-specific threat responses. These are not camouflage patterns, but deimatic patterns, used to ward off less threatening predators from attacking. More interestingly, these pattern transitions are highly deterministic, allowing the observer to determine whether a threat is approaching or receding, depending on the sequence of patterns displayed. We wondered whether cuttlefish camouflage patterns behave in a similar, deterministic manner.

To our knowledge, no attempts have been made to examine cuttlefish pattern transitions. Hanlon and coworkers (1999) have shown that octopus (*Octopus cyanea*) continually alter their patterns while wandering outside their dens. They found that octopus change their patterns at an average rate of 2.95 times per minute. In contrast, Zylinski and coworkers (2009b) suggest that cuttlefish only change their patterning when moving on disruptive backgrounds. They examined how cuttlefish use their dynamic patterning for motion camouflage, and discovered that a cuttlefish displaying disruptive features will reduce the pattern's contrast to camouflage their movement. If the cuttlefish is not displaying disruptive features, it maintains its pattern while moving. When the cuttlefish stops moving it resumes the pattern used prior to moving. We hypothesized that cuttlefish would attempt to remain maximally cryptic, and in conjunction with Zylinski's motion camouflage results, we predicted cuttlefish would move between two different clusters (i.e. patterns) on a given background.

3.2 Methods

Subjects

Cuttlefish eggs were collected from the south coast of England and kept at the SeaLife center, Brighton, UK. Cuttlefish hatchlings (n=10) were fed ad libitum on mysids and naupliids and were kept under a 12-hour light-dark cycle. Subjects were approximately 4 months old at the start of the experiment with an average mantle length of 36.87mm.

Stimuli

We produced three artificial and three natural backgrounds to compare the background variation with cuttlefish camouflage patterning. The artificial backgrounds consisted of black and white checkerboards (3, 8, and 12mm sized checkers). These test stimuli have long standing history of producing the range of uniform, mottle, and disruptive

body patterns. The natural backgrounds comprised of sand, pebbles, and stone, also known to produce the uniform, mottle, and disruptive patterns, respectively. The variation in size, contrast, and depth provide larger statistical differences compared to the artificial checkerboards (fixed to a specific size and contrast).

Experimental set-up and procedure

Ten cuttlefish were tested individually inside a circular arena made of Perspex with a diameter of 22-cm and a height of 10 cm, which was placed inside an experimental tank that minimised disturbance (Kelman et al., 2007). Images were acquired with a Nikon D5000 digital camera. Backgrounds for each condition were placed inside the tank with the Perspex ring sitting on top.

Animals were placed inside the arena with images taken every minute for 30 minutes with a total of 30 images per run, for a total of 1,800 images obtained. Cuttlefish were monitored for signs of undue stress during the trial periods and only handled once per day.

Image scoring and clustering analysis

Images were scored using the handscoring method (Mathger et al. 2006). Each image was scored based on the level of expression of 23 chromatic components. We used 23 of the 34 chromatic components, removing any sexual signaling (e.g. zebra stripes), iridescent, or arm components. Scoring ranged from 0 (not expressed), 1 (lightly expressed), 2 (moderately expressed), or 3 (strongly expressed). Images were cut out and randomised from the background to prevent background biases during scoring. We reduced the 24-dimension space using principal components analysis (PCA) (Kelman et al. 2007) and clustered using a gaussian mixture model (GMM).

We next need to extract the clusters from our principal component data. In the past, a k-means algorithm was used to assign each data point to a cluster (Ghahramani and Beal 1999), however the clusters do not take into account the covariance of the data assigned to each cluster. By implementing a GMM, the assignment of data to a cluster takes the covariance of the cluster into account. Specifically, data points are assigned to clusters based on the probability they are within it, assuming a Gaussian distribution within PCA-space specified by the covariance of the data assigned to each cluster. This means that, a point equidistant (in Cartesian distance) from two clusters, will, for instance, be assigned to the cluster which is less densely packed in the relevant dimensions and so best matches the underlying distribution of the data.

One difficulty with clustering is determining the correct number of clusters. One cannot simply use the ‘best-fit’ of data to cluster centres, as the optimum is simply to have one cluster per data point, overfitting the data. GMM’s prevent this through the use of the Bayesian Information Criteria (BIC) to determine the optimal number of clusters. When adding extra clusters, the log-likelihood may increase, so the GMM uses a complexity penalty to take the higher number of parameters into account for this and prevent overfitting (Chen and Gopalakrishnan 1998).

However, there is still variation within the data. To see the effects of this, we examined the clusters for any biological significance, by examining the range of patterns on each principal component axis, and the patterns closest to and furthest from its respective centroid to visualise each cluster. In other words, we examined both the ‘up’ and ‘across’ patterns furthest from the centroid to encompass the full range of patterns.

To determine how cuttlefish move between these cluster spaces, we counted the number of times cuttlefish transitioned between each of the cluster spaces. These were used to create a Markov model, where the number of transitions was also used to calculate how often they stay within a given cluster or transition between clusters.

3.3 Results

Coordinated Component Expression

The clustering results failed to show distinct boundaries between the clusters (Figure 3.1), suggesting that chromatic components possess a greater degree of independent expression than previously thought. Even so, our model proved insightful in categorising the range of patterns, where we can visualise the patterns along a continuum.

Mixture models produce different results depending on the initial conditions. Our model consistently generated six clusters as the most probable, however we examined the five and seven clustering results for any biological interpretations that may have been missed. For example, none were found to relate in a natural way to the familiar body patterns described by Hanlon and Messenger (1988). Six clusters adequately represented our data in a meaningful way, where each cluster represented a range of patterns.

One important aspect of our model comes from our PC axes, which demonstrate the range of potential patterns exhibited in our body pattern space (Figure 3.2). Low scores on

PC1 show uniform patterns which transition into disruptive patterns on high scores. PC2 shows uniform (low score) to weak mottle patterning (high score), while PC3 shows weak mottle (low score) to strong mottle (high score). When we compare this with the correlation matrix and representative patterns, usually produced by the handscoring method (Figure 3.3), we see the high score value patterns, from our 3D plot, being the representative pattern for a given PC axis in the handscoring method. Unfortunately, the handscoring PCA does not demonstrate the range of patterns with low correlation values on each axis. Using our clustering method on top of the PCA handscoring, we obtain a more robust idea how each cluster varies in our body pattern space.

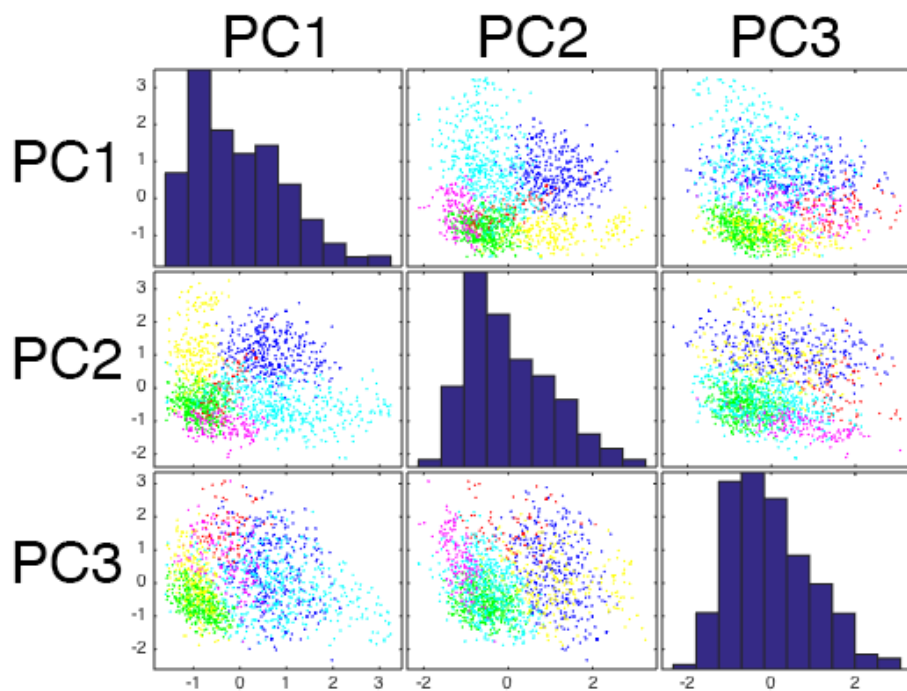


Figure 3.1: Scatter plot matrix showing the 6 clusters in the 3 dimensional PC space. Each row and column shows the colour-coded points for each cluster. Each PC intersect shows a histogram of the density of points on the axis.

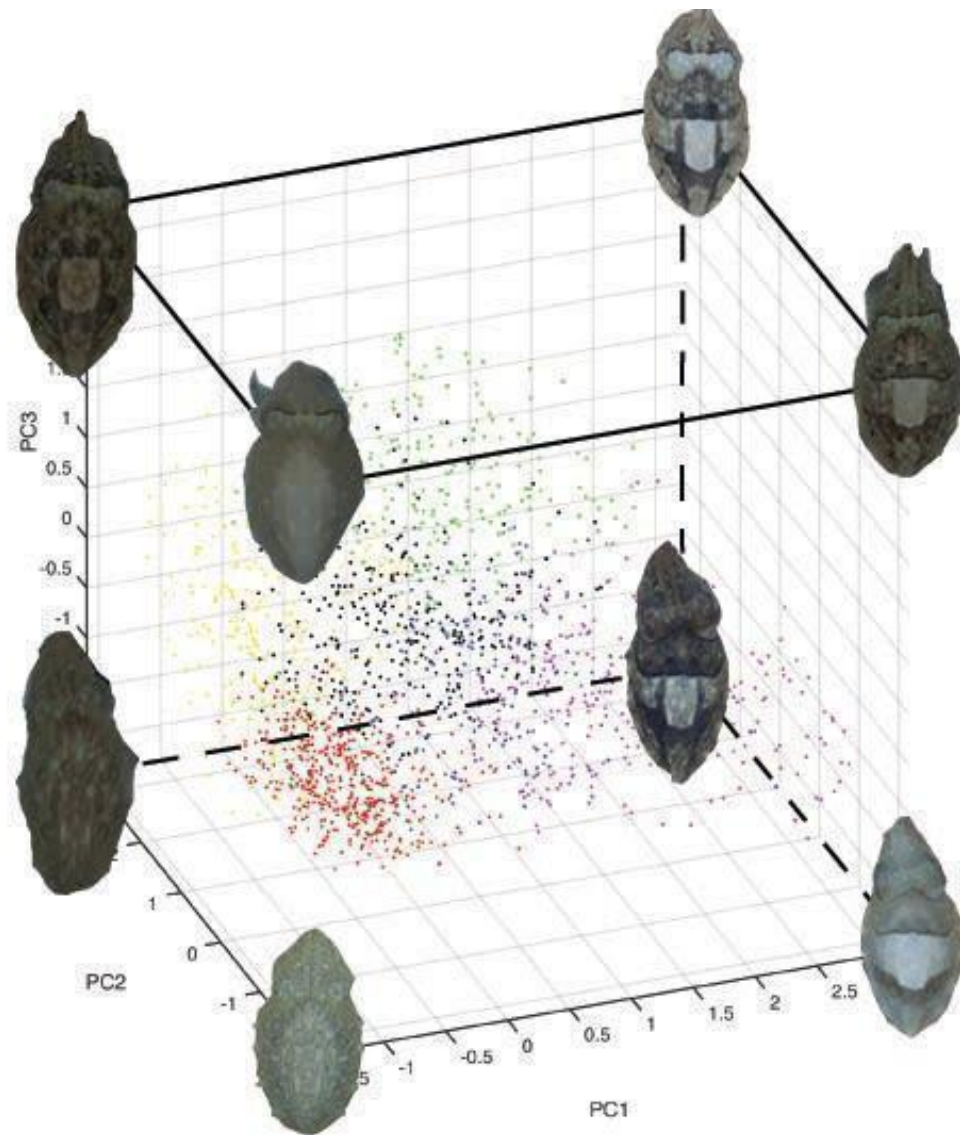
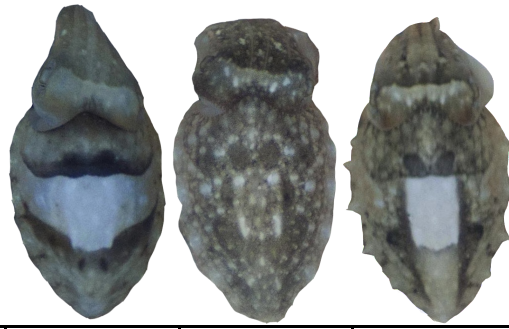


Figure 3.2: The range of cuttlefish patterns at the extremity of each axis in the Principal Component body pattern space. Each point represents an individual pattern with colours representing cluster categories. No boundaries between the clusters demonstrate cuttlefish patterns lie on a continuum. The cuttlefish patterns at the extreme (e.g. -1 to +1) represent the pattern closest to that extreme. This helps visualise how cuttlefish vary their patterns along this continuum, where for example, a low PC1 and PC2 score show 'stipple' patterns, with higher expressions along each axis expressing patterns for disruptive (PC1) or mottle (PC2) patterns.



Component	PC1	PC2	PC3
White posterior triangle			
White Square			
White mantle bar			
White lateral stripe			
White fin spots			
White fin line			
White neck spots			
White landmark spots (arms)			
White landmark spots (head)			
White landmark spots (white square)			
White splotches (mottle)			
White major lateral papillae			
White head bar			
Anterior mantle bar			
Posterior mantle bar			
Paired mantle spots (posterior)			
Paired mantle spots (anterior)			
Median mantle stripe			
Mantle margin scalloping			
Anterior head bar			
Posterior head bar			
Eye ring			
Dark arm stripes			

	+0.40 to +1.00
	-0.39 to +0.39
	-0.40 to -1.00

Figure 3.3: (Top) Examples of body patterns expressing components with high correlation ($>+0.40$) for each Principal Component axis. (Bottom) A correlation matrix, of the 24 chromatic components, for each principal component axis. Loadings are thresholded at 0.4 to allow components making a strong positive or negative contribution to be readily identified (Zylinski et al., 2009b)

By examining the patterns ‘up’ and ‘across’ a given cluster (i.e. the patterns closest to and furthest away from its respective cluster centroid), we can visualise how the cluster classifies each pattern by its visual similarity. Each cluster represented a range of patterns within the uniform, mottle, disruptive (UMD) classification scheme; breaking them up into smaller subdivisions, based not only on the finer divisions, which are consistent with Hanlon and Messenger’s (1988) original classification, but also on the level of component expression (Table 3.1; Appendix 1). For example, cluster 1 demonstrates the mixed type of pattern Hanlon and coworkers (2009) mentioned, where the cluster contains a mix of strong mottle and weak disruptive patterning. We continue to use the UMD classification to illustrate the range of patterns in this cluster, however, the range of components is remarkably similar, with the expression of the white square as the primary exception.

Cuttlefish pattern transitions

To our surprise, cuttlefish did not stay within 2 cluster spaces as hypothesised. Our Markov models demonstrate that cuttlefish freely move between the cluster spaces, favouring some clusters over others (Figure 3.4). The sand and 3mm condition showed the fewest cluster transitions, neglecting the 1st and 6th cluster (both corresponding to disruptive type patterning). As the size of objects on the substrate increased, so did the variation in cluster transitions. Cuttlefish freely move between the conditions, with the only commonality being rare transitions between clusters 4 (stipple and uniform patterns) and 5 (mottle to strong mottle patterns).

Artificial backgrounds elicited the lowest range of transitions, with the animals opting to stay within 3-4 clusters. For all three artificial backgrounds these included uniform, mottle, and disruptive patterns, with the 8mm checkerboard producing the widest diversity. The natural backgrounds showed a wider range of transitions both within the same cluster and between others. Cuttlefish on the sand background favoured uniform and mottle patterning with a 10% chance of displaying disruptive patterning in cluster 3. Unsurprisingly, cuttlefish on pebble and stone conditions favoured disruptive and mottle patterns. It should be noted that the most favoured cluster in each of the artificial conditions corresponded to the pattern expected from previous work (e.g. 3mm elicits uniform patterning; e.g. Zylinski *et al.* 2009b). In the natural conditions, the sand condition, like the 3mm condition, favoured a uniform/stipple cluster, however, the pebble and stone conditions showed more unusual cluster favourability. The pebble condition (usually known to elicit a mottle patterning)

produced a strong disruptive pattern, whereas the stone condition (usually known to express strong disruptive patterns) gave a cluster ranging from strong mottle to light disruptive.

At the population level, our results demonstrate that cuttlefish display patterns from all six clusters (with the exception of when on sand using four clusters). We wondered what level of individual variation existed within this and whether every individual expressed the full range of patterns. We discovered that an individual would display an average of three patterns across all conditions. The range of patterns for an individual varied from 2-6 clusters, with the exception of sand capping the range at 4 clusters. This demonstrates that an individual only expresses up to half the potential patterns demonstrated at the population level.













Cluster #	Description	Closest to centroid	Furthest from centroid
1	Strong mottle to weak disruptive		
2	Weak mottle to avg mottle		
3	Weak banded disruptive to strong banded disruptive		
4	Stipple to uniform		
5	Mottle to strong mottle		
6	Strong disruptive		

Table 3.1: The 6 clusters generated through the GMM. The table lists the cluster number, a visual description of the patterns, and the pattern closest to and furthest away from its respective cluster centroid.

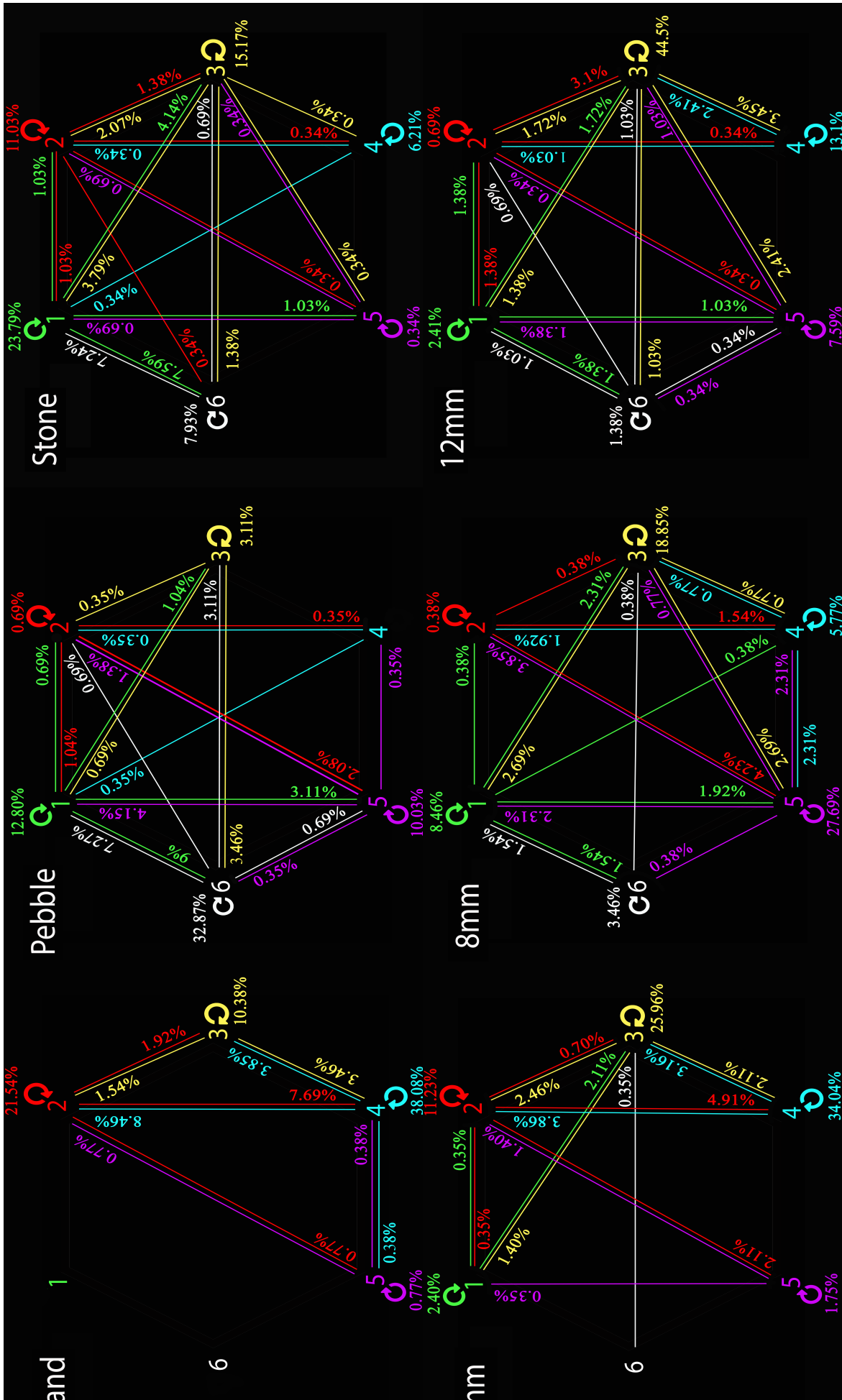


Figure 3.4: A markov model demonstrating the probability a cuttlefish transitioned within or between a cluster. Each line corresponds to a transition from the cluster of the same colour.

3.4 Discussion

Coordinated Component Expression

Our clustering method finds about six distinct clusters in the cuttlefish patterns, but within the 3-D PC space used there are no distinct boundaries between cluster groups (Figure 3.1). This suggests component expression is more independent than once believed, and that the total combination of component expression across pattern types provides a rich continuum of patterns. We can better visualise the clusters and the patterns encompassed in our body pattern space when we plot our data across all 3 PC axes (Figure 3.2). In this 3-dimensional space, we can visualise the range of patterns and how they vary across each axis. Each of the traditional UMD patterns is present, such as uniform (low PC1, low PC2, high PC3) and its variant stipple (low PC1, low PC2, low PC3), but more interestingly, we see that high PC1 expressions demonstrate a wider range of disruptive patterning, with a diversity of components accompanying the white square and white mantle bar. When we compare this plot to the traditional PCA correlations and their representative pattern (Figure 3.3), each of the representative patterns is present in our 3D plot, however we miss a lot of variation of low-correlated patterns or any variation between PC scores.

Although the range of patterns in a given cluster lies in 3-dimensions, we examined the up and across variation (Appendix 1) to visualise the range of patterns within. Interestingly, the range of patterns in each cluster can be aligned, similar to the UMD classification. The patterns align to create a continuum of patterns based on the spatial scale of the pattern components, as well as, their level of expression. This continuum ranges from the uniform pattern (little to no components expressed) through to the opposite extreme with the disruptive categories (large squares and bars across the body). This is most evident in cluster 1, showing the transition from mottle to disruptive categorisation, where both patterns contain a similar expression of components, with the high expression of the white square defining the disruptive pattern.

This is not to say the UMD classification is not useful. We know from previous research that the spatial scale of objects in the environment plays a major role in the selection of pattern type and the UMD classification categorises these patterns based on gross-scale changes along the mantle. What our research suggests is that the amount of diversity within a given pattern type is more varied than once believed. This is why we suggest a revised pattern categorisation that encompasses the level of expression of components to provide

weak (i.e. low component expression) or strong (i.e. high component expression) variations of a pattern category (Table 3.2). Component expression plays a role in the level of contrast on a given pattern and our current understanding of cuttlefish pattern selection suggests background contrast also plays a significant role in pattern selection (Chiao et al., 2015).

More research is necessary to understand the degree of independence for a given component. We suggest future research should focus on the relationship of component expression, not only for presence of a component [similar to Crooke and coworker's method (2002)], but also for the level of expression. Other features of a given component may be needed, but these two variables have proven useful in teasing out a more robust understanding of cuttlefish patterning. Another point of interest would be to include a range of social patterns in the clustering space to determine if chromatic components possess similar independence in social signals. Camouflage patterns need to blend to a wide range of backgrounds, whereas social patterns need to stand out. The range of components on social patterns are more defined and less varied, but may possess enough similarity, such as the case of Crooke and coworker's results (2002), to also lie on a continuum.

Cuttlefish pattern transitions

We found no evidence that cuttlefish camouflage patterns follow any sequential or deterministic patterning, unlike deimatic responses (Langridge et al. 2007, Langridge 2009). Cuttlefish were found to vary their camouflage patterns, encompassing all the clusters, with the exception of sand, which displayed patterns in only 4 clusters (Figure 3.4), and transitions between clusters were stochastic, depending on the probability of the expression of a given pattern. We are able to suggest that this characterisation is size dependent, where the 3mm and sand conditions showed less variation in cluster transitions compared to the other conditions. While cuttlefish may transition or employ patterns across all cluster spaces at the population level, we discovered that individuals average 3 cluster spaces per condition. This implies that an individual may vary between 3 clusters spaces, but the variation between individuals differs enough to show our cluster variation encompassing all 6 clusters at the population level.

When we consider the amount of time cuttlefish spent within a cluster, we do see some clusters favoured over others in each condition. For the artificial conditions, the 3mm, 8mm, and 12mm selected clusters using patterns we commonly associate to each background (e.g. uniform, mottle, and disruptive respectively). The natural patterns failed to show this

consistency. The sand condition favoured the uniform pattern, as expected, yet the pebble and stone conditions did not. We expected the pebble to favour a cluster with more mottling, yet they highly favoured strong disruptive patterns in cluster 6. The stone condition, which would suggest a strong disruptive category (cluster 6) gave patterns more often in cluster 1, showing a range of strong mottle to weak disruptive. The size of our cuttlefish may have played a role, where the pebbles more closely matched the size of the white square compared to the stone condition (Chiao and Hanlon 2001).

Even though the cuttlefish favoured a set of clusters, only 24%-45% of the time was spent in these favoured clusters. Hanlon and coworkers (1999) observed a similar behaviour in wild octopus species, where the octopus continually altered their appearance while outside their dens. They proposed two possible reasons for this behaviour. The first proposed that octopus camouflage is neurophysiologically expensive. Cephalopods use vision for multiple tasks (e.g. foraging, memorising the seascape, etc.), where it might be necessary to use less effective camouflage patterns to attend to other tasks. The alternative explanation proposed was that the octopuses might benefit from using diverse patterns to obstruct predator search recognition, sometimes known as protean behavior (Blough 1992; Dukas and Kamil 2001). This explanation follows the search-image hypothesis, where rarer phenotypes are predated upon disproportionately less frequently to the more common phenotypes. The underlying principle being that the predator creates a template of previously encountered prey patterns, which essentially trains the predator to locate the trained (i.e. more common) phenotypes.

Our lab-raised cuttlefish were not tested in an expansive environment to that of the wild octopus, removing the need to spend large amounts of time foraging or memorising the seascape. This supports Hanlon and coworkers second hypothesis for polyphenetic camouflage. When we consider the individual variation across each condition, each cuttlefish selects its own preference of around 3 patterns. With the population level encompassing all the cluster spaces, perhaps some individuals benefit from displaying the rarer morphs compared to the most common morphs. It should be noted that Hanlon and coworkers discovered octopuses living in environments with more predators camouflaged themselves more often. Our cuttlefish have never been exposed to any predators, possibly influencing the high rate of pattern changes. Our evidence cannot fully support the search image hypothesis, as we have not been able to demonstrate that the rarer phenotypes benefit from the more commonly used patterns. What we can say with certainty is that cuttlefish, like octopus

camouflage, continually alters its appearance on a given substrate and the patterns do not follow any sequential expression.

Our research supports the notion that component expression is more independent, allowing a wider range of pattern variations than previously believed. We were also able to demonstrate that cephalopods continually alter their appearance and this does appear to be influenced by the spatial scale and contrast of objects on the substrate. What this means in the broader context for camouflage efficacy remains to be known. However, we were able to use a GMM to successfully tease out a new pattern categorisation based on the coordinated expression of the components. Machine learning methods are slowly entering the biological realm, proving to be useful, if difficult tools to implement. However, our research has shown that these methods can tease out discrete categories, as little is known how the components coordinate with each other to create the full repertoire of possible patterns. This has allowed us to propose an updated classification scheme where the major pattern classification (e.g. uniform, mottle, and disruptive) can be subdivided into groups based on the level of expression (i.e. contrast) of the constituent components.









Uniform	Stipple	Weak mottle	Strong mottle
			
Weak disruptive	Strong disruptive	Weak banded disruptive	Strong banded disruptive
			

Table 3.2: Proposed pattern categorisation scheme demonstrating the continuum of patterns (from top-left to bottom-right) based on spatial scale and contrast of the patterns.

4. Decoding cuttlefish (*Sepia officinalis*) communication using human speech recognition models

4.1 Introduction

Most people are familiar with the common cuttlefish (*Sepia officinalis*) only from finding their cuttlebones on the beach or in the cages of budgerigars, but they are one of the most remarkable creatures in the animal kingdom. They cannot see colour, but can both see the polarization of light, and generate highly coloured displays (Mathger et al., 2009). They may be able to perform imitation learning (Huang & Chiao, 2013), but fail to recognise other individuals (Boal, 1996, 1997, 2006). Their most spectacular ability though is their ability to generate, almost instantaneously, highly variable coloured patterns across its body (Hanlon and Messenger 1988). It uses these patterns both for camouflage and for communication.

Most work on these displays has used them as a way of probing the cuttlefish's perceptual system (Chiao and Hanlon 2001; Mathger et al., 2006; Zylinski et al., 2009a/2012). The principle underlying this work is to test the visual parameters or features that the animal can discriminate: if a cuttlefish displays a different pattern on its body when presented with two different visual inputs, then this implies that their perceptual system can distinguish between these patterns. Here, in contrast we look at these displays in terms of their role in communication, and in particular, whether this communication is language-like.

Defining communication, in the sense of Shannon, is simple (Shannon and Weaver 1949). Some information source (say a cuttlefish), generates a signal. This signal is transmitted through a channel (the pattern of light reflected by the animal), possibly corrupted by noise, and then detected by a receiver. Based on this signal, the receiver updates its state of knowledge (and hopefully its behaviour so we can measure this). The (average) difference between the uncertainty in behaviour before and after receiving a message, quantified by entropy, measures how much information is transmitted.

This model of communication is very general, and both communication in cuttlefish and humans can be quantified by these methods, but so can very different systems, such as modems and black holes (Hawking 2005). Therefore, rather than simply using information

theory, we also attempt to decode these signals, not using general purpose methods, but the methods that have proved successful in interpreting spoken speech (Abushariah and Gunawan 2011).

Spoken languages vary enormously, and the information conveyed can vary between the works of Shakespeare to the gentle noises used to sooth a crying child, but all follow a certain structure. Out of the potentially infinite variety of sounds, 1) a small number of continuous dimensions [such as formant location (i.e. peak amplitude), intensity or duration] are used to signal, 2) a set of discrete phonemes (an abstract unit of morphological analysis in linguistics, that roughly corresponds to a set of forms taken by a single word). 3) These phonemes are continuously and flexibly inflected (varying say the stress, pitch or tone). Lastly, 4) these inflected lexemes are strung together in non-random sequences (syntax): In response to the baby's crying, the mother generates one of the potentially infinite range of sounds that would be identified with the label voiceless palate-alveolar sibilant (the sound shh); modifies it to be longer but quieter than normal; then repeats this until the baby stops crying.

Our basic hypothesis is that this four-level structure is common to many other animal communication systems, and to cuttlefish signaling in particular. Signaling in cuttlefish may or may not be language (which is perhaps a philosophical problem), but could be deemed to be language-like if we get meaningful decodings by directly applying the methods that have proved successful to understanding human speech to cuttlefish signals. Such a decoding will produce a sensible interpretation of essentially all human languages, but unlike information theory, would not produce sensible decodings when applied to the output of a modem, or the radiation produced by black holes.

In particular, we show that visual signaling in cuttlefish, achieved by dynamically and continuously changing the coloration patterns on their body is directly decodable using the highly developed human speech recognition techniques. Based on an analysis of 796 example cuttlefish signals, we show this strategy of simply adapting existing speech recognition techniques to cuttlefish communication works well, and in this sense the signals can be called language like.

4.2 How to decode human speech (and how to apply it to cuttlefish)

Until the recent development of deep neural network methods (which are very data hungry and hence not applicable here), almost all speech recognition systems work by applying the same five steps.

Firstly, the raw input is filtered by a bank of frequency tuned filters. Sometimes the form of these filters is based on the known properties of the ear (using say a Gammatone filter bank, Slaney 1993), but more often a local Fourier transform is used. Here, after warping the images of the cuttlefish to a standard reference frame (see Appendix 2) (Anderson et al. 2003) we use a spatial frequency tuned log-gabor filter bank (Field, 1987), again because it approximates early (visual) perceptual processing). There are though two (minor) complications. Firstly, sound is a one-dimensional signal and an image is a two dimensional one. We therefore extracted the local energy at four orientations (vertical, horizontal and 45 degrees), and four scales. Secondly, since Helmholtz, it has been believed that humans are insensitive to the local phase of sounds (but see Paterson 1987). In vision this is not true both for humans (Oppenheim and Lim 1981), and for cuttlefish (Zylinski et al 2009a). We therefore supplemented the local power of the filter output by the (cosine and sin of) the phase.

The next stage of analysis is to summarize these measurements in a way that means that the summarising numbers are approximately independent. Most methods exploit the harmonic structure of speech signals, and therefore use a cepstrum based representation (Bogert 1963). Images will not have this harmonic property so we therefore simply summarise the filter outputs using principal components analysis (which also has the approximate independence property). We choose to only extract three components given that few studies show that three components are required to capture the variation in cuttlefish patterns (Kelman et al. 2007), but believe there may be meaningful information in higher components given a larger data set. Unfortunately, our method for identifying clusters is essentially a (Bayesian) density estimation technique. Density estimation suffers the curse of dimensionality: the amount of data required scales to the power of the number of dimensions (Friedman 1987). This means that despite the fact that there may be additional information in the higher components, it is better to reliably extract most all of the information in three

dimensions rather than unreliably extract it in many. Given a (much) larger data set, we should be able to explore information in these higher components.

We next need to extract clusters in this low dimensional summary (for speech these relate to the phonemes). Historically this has been achieved using the crude k-means algorithm, but recent models fit a mixture of Gaussians model. We again use a mixture of Gaussians, but instead of simply maximising likelihood, by using a variational approximation to the full Bayesian solution (Ghahramani and Beal 1999), we can infer 1) the most probable number of clusters in the data; 2) the means of these clusters; 3) the structure of the variation within each cluster (how the basic token can vary across different uses, captured in our model in terms of a factor analysis model). Lastly the model provides estimates of the posterior probability that each of our 796 images came from each cluster.

Again there is a complication: cuttlefish are known to be able to generate separate signals on each half of their body. We therefore analysed separately the left and right halves of the body. This therefore acted as a limited test of reproducibility.

The next stage in speech recognition is to exploit the temporal structure (usually using a hidden Markov model). Here, since we only have static images, we ignore this aspect of signaling. It should however be noted that changes in coloration to approaching or receding threats follow a fixed sequence related to the change in threat level (Langridge et al., 2007; Langridge 2009).

The last and least developed stage in speech recognition is to exploit semantic context. The most successful method is Googles word2vec method (Mikolov et al. 2013). This infers semantics by regressing a given signal to the context (surrounding words) it was generated in. We do not have this information so we performed (cross validated lasso multinomial) regression (Tibshirani 1996) of the cluster associated with a given signal with a crude vector representing the context the cluster was generated in. This context was represented by the following fields: Age (old or young), Sex (male, female, or unknown), Social situation (yes or no), under stress (yes or no), and since we suspect age interacts with the use of social signaling, we also entered a social/age interaction term.

4.3 Results

We ran the variational Bayes mixture of factor analysers model on both the right and left halves of the body pattern. Like many mixture model based methods, this method gives different results dependent on initial conditions, so we ran the model 100 times and found the model of the highest estimated evidence (sometimes known as the marginal or type two likelihood). Two solutions consistently emerge with very similar evidence: there were either ten or eleven separate clusters with 10 being slightly more probable for the left half and 11 for the right half (Figure 4.1). Inspection of the solutions (see later) showed that the solutions were essentially identical save that it was ambiguous whether a cluster responsible for the uniform pattern was split into two or not. For simplicity, we therefore present the results for the 10-cluster solution (Figure 4.1).

This shows that the patterns displayed by the cuttlefish naturally fall into a number of discrete words, but does not show that these words represent separate signals. To show this, we then calculated the information present in these signals about the context they were generated in. Note that this context was not used in identifying the clusters so this represents an unbiased estimate. We found that that cluster identity provides 1.6 bits of information about context. If each cluster was equally probable, then the maximum information that could be communicated would be 1.6 bits: most of the information about context is communicated by these signals. This rate of information is also comparable with that of a single letter in English (1.2 bits to 1.7 bits- Shannon (1951)), the difference being that letters can be combined to form words: with cuttlefish, the distinction between phonemes and words does not seem to exist. Note that there is another similarity to the statistics of English (and all other languages): to first approximation the signals obey Zipf's law: the frequency of occurrence of a word is inversely proportional to the rank (i.e. frequency of occurrence) of the word (Piantadosi 2014).

Information theory shows that the signals contain information about the context they were generated in, but not the nature of this relationship. We therefore attempted to gain insight into the nature of the clusters by finding the most probable examples of each cluster. Most of these clusters made intuitive sense and correspond to body patterns that are commonly classified by eye, but two patterns did not correspond to standard classifications. In particular, the two light disruptive patterns would be normally classified as identical (Figure 4.2). Our analysis reliably identified them as separate signals. Which is correct?

As a first step to answering this question, we therefore performed Lasso based regression, attempting to predict the cluster generated from the context it was displayed in. The results are shown for the left half in Table 4.1. Firstly, as can be seen, each signal was generated in very different contexts. Secondly, it is clear that the solution given by the model is the correct one: though the two signals look to the ‘non-native’ speaker as identical, they are generated in very different contexts. The classification provided by applying speech recognition methods is superior to that provided by an observer.

What is the nature of the difference? To find this out we simply averaged all the signals in the two visually similar but contextually different signals, and subtracted these means (Figure 4.2). As can be seen, though subtle, there are reliable differences in the degree and location of the expression of parts of the body: the fact that these differences look visually similar to the ‘non-native’ speaker does not mean they are the same

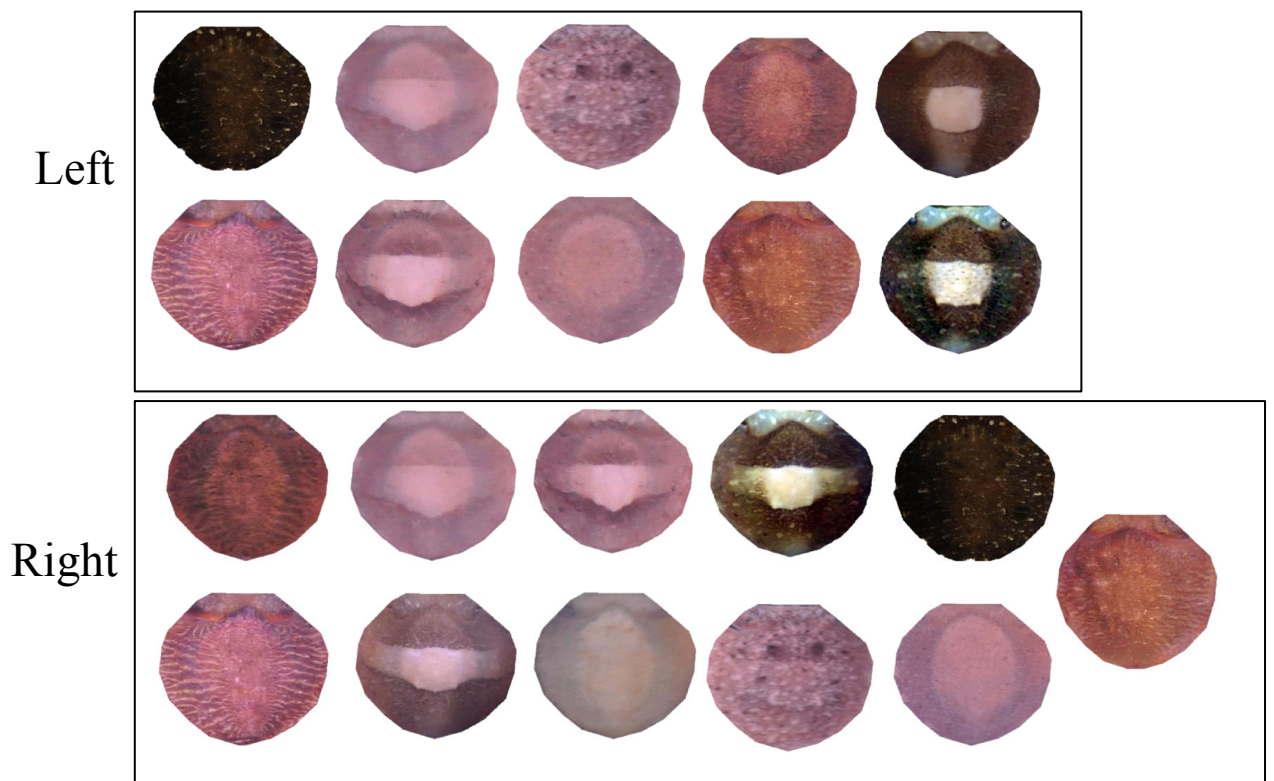


Figure 4.1: The left (upper) and right (lower) half cluster representations produced by our variational Bayes mixture of factors analysis.


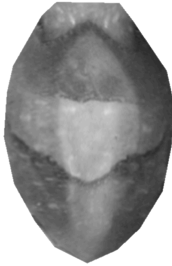
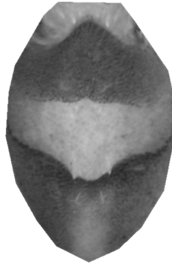
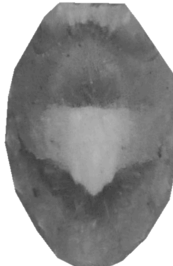
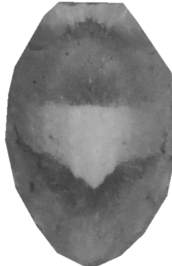

	Cluster representation	Example 1	Example 2
Light Disruptive 1			
Light Disruptive 2			

Figure 4.2: Examples of the within cluster variation for the 2 visually similar clusters. The cluster representation generated by our model might not account for the variation inherent within the clusters themselves. The examples show little variation within the clusters, demonstrating greater visual similarity between the two clusters.

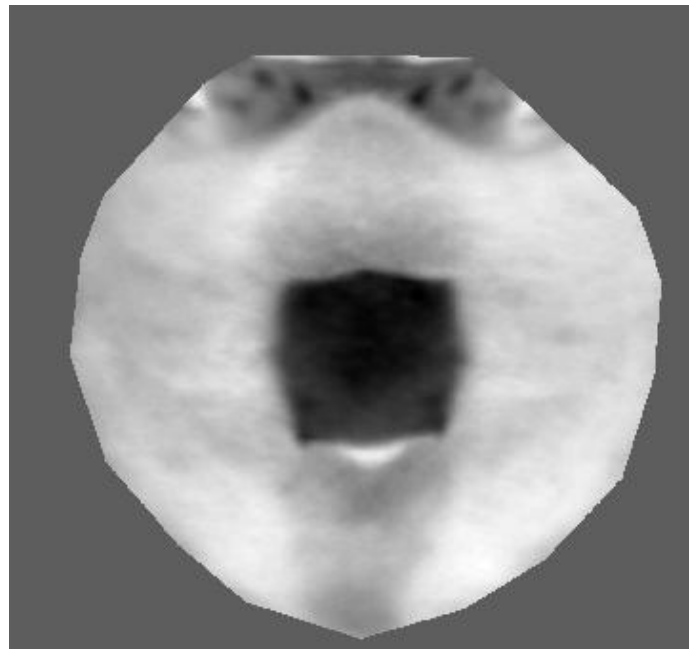


Figure 4.3: The difference image obtained after subtracting the mean of each cluster from each other. The darker the area on the pattern indicates increased similarity between the two clusters. While the two patterns appear visually similar to the observer, by subtracting the two

images, we are able to see how the patterns differ. These subtle differences might not be obvious to human observers, but may be salient features cuttlefish easily distinguish.










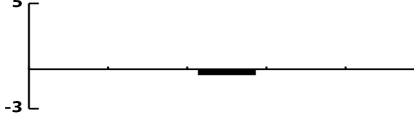

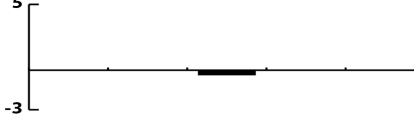





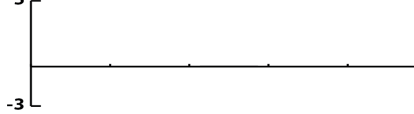


Name	Example	Regression Coefficients	Definition
Strong zebra			An adult-only, male aggression display toward rival males or other conspecifics
Weak zebra + juvenile uniform			An adult-only male and female aggression display. Juvenile pattern used on light coloured substrate.
Splotch			Female aggression display. Thought to be female-female specific.
Light uniform (juvenile)			Camouflage pattern commonly used on light coloured substrates (e.g. sand) and social juvenile interactions
Dark uniform			Camouflage pattern commonly used on darker substrates (e.g. dark sand or mud)
Mottle			Camouflage pattern commonly used when medium sized particles on the substrate (e.g. small pebbles or shells)
Light disruptive (juvenile)			Camouflage pattern used with larger objects on light coloured substrate. Sometimes used in social juvenile interactions.
Light disruptive (adult)			Camouflage pattern used when larger sized particles on light coloured substrate (e.g. large stones or on sand)
Dark disruptive			Camouflage pattern commonly used when larger sized particles on dark coloured substrate
Dark disruptive			Camouflage pattern commonly used when larger sized particles on dark coloured substrate

Table 4.1: A cuttlefish dictionary of our 10 clusters with the names of each pattern, based on Hanlon and Messenger’s (1988) classification scheme; a representative example of each pattern; the context of each pattern from our logistic regression coefficients, and a definition of the pattern.

4.4 Discussion

We have found that simply applying speech recognition methods that have proved successful to human speech, we get sensible and insightful decodings of the communication system of an animal that is about as different from us as it is possible to be. We found that this decoding extracted a lot of the information about the context in which the signal was generated. We found advantages for this automated system: signals that to the human observer looked very similar were reliably classified as different by the Bayesian mixture model. When we looked at the context in which these patterns were generated, it was clear the model was correct. When we used a subtraction method inspired by fMRI analysis (Logothetis 2008), again subtle but systematic differences were found. In short, we can get a good understanding of different animals signaling system simply by applying standard speech recognition techniques to the signals generated.

There are though limitations to these results. Firstly, though our data set is large for an animal communication experiment, and represents many man-years work from a number of labs, compared to the size of data sets usually analysed in speech recognition training it is minuscule (often these data sets consist of many millions of examples) (Hinton et al. 2012). A large determinant of recognition is simply the size of the training corpus. Again these displays were recorded exclusively in laboratory aquariums. There may be a number of other displays where the conditions required to elicit them just don’t occur in this controlled but unrealistic environment. Luckily, cuttlefish are highly photogenic, and many divers take pictures of them. The image quality required for our method is not great. We therefore hope to set up a web site to allow us to crowd source a data set of examples of signals both an order of magnitude larger, and taken in more naturalistic conditions.

Secondly, whilst it is legitimate to ignore colour of the displays [since other cuttlefish are blind to this (Marshall and Messenger 1996)], we also ignore any structure in the polarization domain. We know that cuttlefish can both adaptively change the degree of polarization of their signals, and can perceive this (Chiao et al. 2007; Mathger 2009; Temple

et al. 2012). Our methods could equally be applied to polarization signals, we simply want to point out that we are possibly only looking at part of the channel that cuttlefish use to communicate.

Lastly, our method not only extracts clusters, but identifies the dimensions of continuous variation within each pattern. The Bayesian generative model assumes that each discrete cluster is modelled by a factor analysis model (Ghahramani and Beal 2000): we have the continuous degree of intonation of each signal. We also have evidence, at least for some of the clusters, that this continuous variation carries information: for instance, the contrast of the zebra pattern, is an honest signal (Boal 1997; Adamo & Hanlon 1996). For this to be usefully decoded, as well as categorical descriptions of context, it would be very useful to have continuous descriptors. These papers show how one descriptor, an indicator of signal of agonistic intent, relates systematically to the contrast (the first factor) of the zebra pattern cluster. Our method automatically extracts not only the qualitative ‘between signal’ structure, but also extracts the quantitative ‘within signal’ structure. This opens up the possibility of exploring intonation in cuttlefish, which we will explore in future work.

There are systematic differences between human spoken language and the signaling of cuttlefish. We have not investigated the distinction between phonemes and lexemes. Though the information content of a single signal is comparable to that of a single letter, and cuttlefish do make non-random sequences of such signals, it seems unlikely that they have anything even approaching the sophistication of human syntax. Despite this, the range of things that needs to be communicated is much smaller than for humans. That the communication system of a creature more similar to a snail than a human being can be successfully decoded by simply using speech recognition techniques means that it may or may not be a language, but it is at least language like.

5. Banded display social signals of the cuttlefish (*Sepia officinalis*)

5.1 Introduction

Cephalopods are renowned for their ability to change their coloration (Hanlon and Messenger 1988). This remarkable behavior is well documented for the European cuttlefish, *Sepia officinalis*. Hanlon and Messenger (1988) described some 54 behavioural components that cuttlefish can express selectively. The expression of these components is coordinated to produce about 13 distinct body patterns, six of which are displayed chronically, for camouflage, and seven more transiently for communication with other cuttlefish and potential predators (Hanlon and Messenger 1988; Langridge et al. 2007, Langridge 2009). Except when spawning, cuttlefish are thought to be primarily solitary (Hanlon and Messenger 1996; Palmer 2006), however, *S. officinalis* utilize three classes of patterns when socially interacting, namely mating patterns, agonistic displays, and deimatic behaviors.

Cuttlefish primarily use their patterns to identify males and females, however studies suggest that females also use olfactory cues to identify potential mates (Boal 1997;2006). Some cuttlefish species, such as *Sepia apama*, use mating patterns in courtship between males and females and to ward off rival males (Hall and Hanlon 2002). In the case of *S. officinalis*, patterns are not used as a mating courtship, but rather to ward off potential suitors.

When two competing males encounter each other or the female decides not to mate, they express agonistic patterns toward the conspecific. Two males display the Intense Zebra Display, the most typical male-male agonistic display, to warn the other male; sometimes this escalates to fighting and potentially harming one of the cuttlefish (Hanlon and Messenger 1988). The Intense Zebra Display consists of dark zebra stripes across the mantle with the fourth arm extended outward, commonly toward the rival male or female. Females also adopt this pattern, but do not extend the fourth arm to either males or females (Adamo and Hanlon 1996). Typically, the larger males dominate, establishing size-based hierarchies, with the larger males copulating more frequently (Boal 1997).

Should the female not accept the male as a mate or a male decide not to engage another male, cuttlefish use deimatic displays to escape. Cuttlefish mate by grasping the female from the front in a head-to-head position and transfer their sperm packets (Hall and

Hanlon 2002). Females not wanting to copulate may be injured during this encounter and attempt to escape to avoid this potential harm. Deimatic displays are meant to startle a predator or rival cuttlefish or in this context, a larger, rival male or unwanted male suitor, such as the ‘intensify pattern’, where the disruptive display darkens (Langridge et al. 2007). Alternatively, the cuttlefish may distance itself from the rival and display an all-dark pattern to help the cuttlefish hide from the rival. If the rival cuttlefish persists, the cuttlefish may ink before jetting away and attempt to camouflage itself (Langridge et al. 2007). The intense zebra and also the eyespot pattern, which is directed at potential predators, can be expressed asymmetrically on the side of the body facing the intended receiver (Hanlon & Messenger 1996; Langridge 2006).

Hanlon and Messenger’s (1988) inventory of the behavioural components and body patterns underpins subsequent work on adaptive coloration in *S. officinalis*. There have been few reports of new components or body patterns in *S. officinalis* (Shashar et al. 1996), but Palmer and co-workers (2006) described a pattern that adult females direct at other females, which may reduce agonistic encounters. The pattern consists of irregularly shaped light and dark spots distributed across the mantle. The anterior third of the body is a darker than the posterior two-thirds. The arms display dark, horizontal bands with white spots distributed across the face and arms. While piloting hypotheses for social interactions between adult male and female cuttlefish, we discovered 2 previously undescribed body patterns that adult female *S. officinalis* direct at conspecifics, which are visually similar to the pattern described by Palmer and others. We suggest that these displays form a set of social signals used by females to conspecifics.

5.2 Methods

Subjects

The pilot study was conducted in the Autumn of 2013, and used five 15-month old, adult cuttlefish (3 male, 2 female) with mean mantle length of 83mm. Cuttlefish eggs were collected from the south coast of England and were kept at the SeaLife center, Brighton, UK in which they were hatched. Cuttlefish hatchlings (n=10) were fed ad libitum on mysids and naupliids and were kept under a 12-hour light-dark cycle. Sex of the cuttlefish was determined by the level of expression of sex-specific patterns, such as the intense zebra, an exclusively male pattern.

Testing apparatus and experimental chamber

In a test, two animals were randomly placed in separate chambers of a T-shaped Perspex test apparatus. There were three 200 x 200 mm chambers, two side by side and the third forming the leg of the T, so that it had a 100mm border with each of the other two. This apparatus was placed into a tank (910mm x 750mm x 1960mm) filled with seawater, with a laminated flat grey substrate to remove any environmental influence. An angled mirror was placed on one side with a small slit opposite, to capture the cuttlefish interactions without disturbance from the observer (Kelman et al. 2007; Zylinski et al. 2009a).

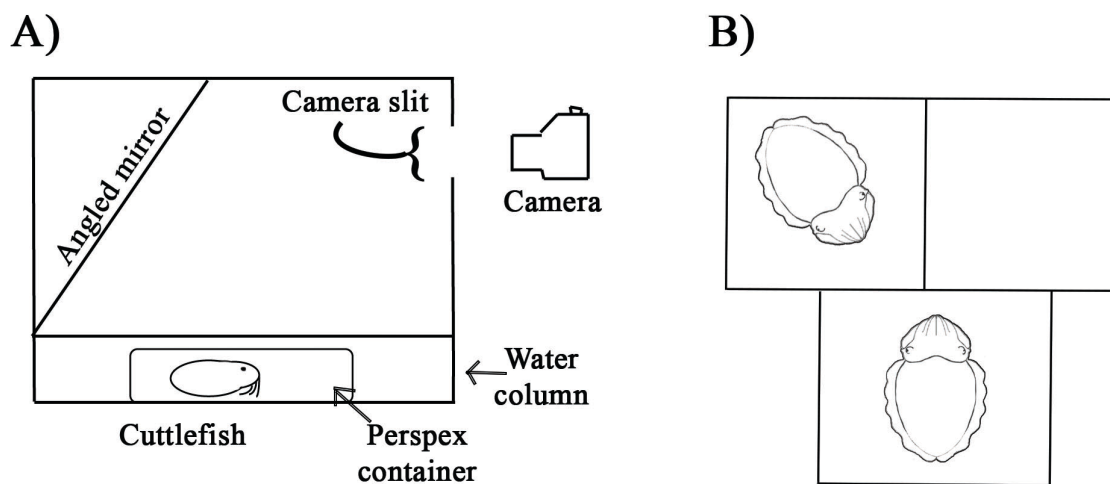


Figure 5.1: A) A side-view illustration of the cuttlefish experimental tank. Cuttlefish are placed inside the t-shaped perspex container, with an angled mirror placed in the tank. The camera is positioned outside the tank, viewing the mirrored reflection of the cuttlefish, preventing the cuttlefish from viewing the observer. B) An overhead representation of the t-shaped perspex container used to examine cuttlefish social interaction. Two cuttlefish were randomly placed into 2 of the 3 compartments.

Procedure

Each cuttlefish was tested with the other available cuttlefish, for a total of 10 trials (Male/Male = 3; Female/Female = 1; Male/Female = 6). Cuttlefish were placed in two of the three compartments, and hence either fully adjacent or sharing a half-length boundary. The transfer time was less than 30 seconds between home container and the experimental apparatus, which is routine in our experiments and causes no discernible stress or harm. Just before the second cuttlefish was placed into the experimental apparatus, a video camera began recording the interactions. A cuttlefish was only tested once per day. Each run was

recorded up to 30 minutes, unless a cuttlefish inked in the container. The video was examined by a single researcher (JJC).

Ethical considerations

These experiments were done in 2013, as a preliminary study of social interactions amongst sexually mature animals. It is believed that cuttlefish are normally solitary, but in aquaria they are often kept in groups, or sometimes in individual tanks separated by transparent partitions, so they are in social contact. Cuttlefish that live in visual contact, do not display the negative responses we report here amongst animals that had been kept in separate tanks with opaque walls, and hence were socially isolated. Consequently these responses were unexpected and novel. Further work of this kind would require appropriate ethical approval. Nonetheless animals were continuously monitored. If they inked, they were removed immediately from the experimental set up and both participants returned to their home tank without delay. The cuttlefish remained in good health without detectable long-term effects, and we believe that the findings are worth reporting. Possible stressful events (e.g. striking attacks or close proximity) elicited threat displays. If they did not ink, the Perspex partitions prevented cuttlefish from physical contact, but (occasionally) allowed them to strike toward one another. Normally, a cuttlefish moved as far possible from the conspecific and then rested.

5.3 Results

The experimental apparatus placed the cuttlefish in close proximity, albeit separated by transparent barriers, which led to conflict. Our initial investigations failed to demonstrate any significant social interactions with all but three observations resulting in immediate inking. From three trials, two lasted 10 minutes (one male/female, one female/female) before inking, and the other lasted the full 30 minutes (male/female). In each of these three trials the females displayed two previously unreported patterns and behaviour, none of which were observed in the control conditions.

The 'Banded' pattern and 'Banded Strike' pattern are acute displays, lasting a few seconds. (Hanlon and Messenger 1988). We now describe each pattern, in the convention of Hanlon and Messenger (1988), detailing the behavioral interactions between the two cuttlefish. Due to the low resolution of our video camera, postural, textural, and locomotor

components were not considered, making the comparisons distinctly chromatic, requiring further analysis to fully define these other characteristics to fully define the body patterns.

Banded pattern

The ‘Banded’ pattern consists of a pale uniform background, with darkened anterior transverse mantle line, prominent band just above the anterior transverse mantle line (hereafter referred to as banded mantle bar), and the posterior head bar (Figure 5.1). In this study, only females expressed it. The cuttlefish frequently displayed only a portion of the pattern at a conspecific (i.e. directional, asymmetrical patterning). Of the fifty minutes of video recorded, the Banded display appeared 15 times, for 2-10 seconds. It was apparently a defensive response to conspecific presence or aggression. Attempted attacks and aggressive patterns (e.g. intense zebra display or darkened head and arms; Adamo and Hanlon 1996) by the conspecific always provoked the pattern ($n = 9$). The display also occurred when the other individual ($n = 3$) or before the 'Banded' cuttlefish ($n = 3$) retreated. In response to the Banded Display, conspecifics continued to attempt attacks ($n = 3$), attempted to flee ($n = 3$), displayed agonistic patterns (e.g. intense zebra or weak zebra) ($n = 6$), or did nothing ($n = 3$).

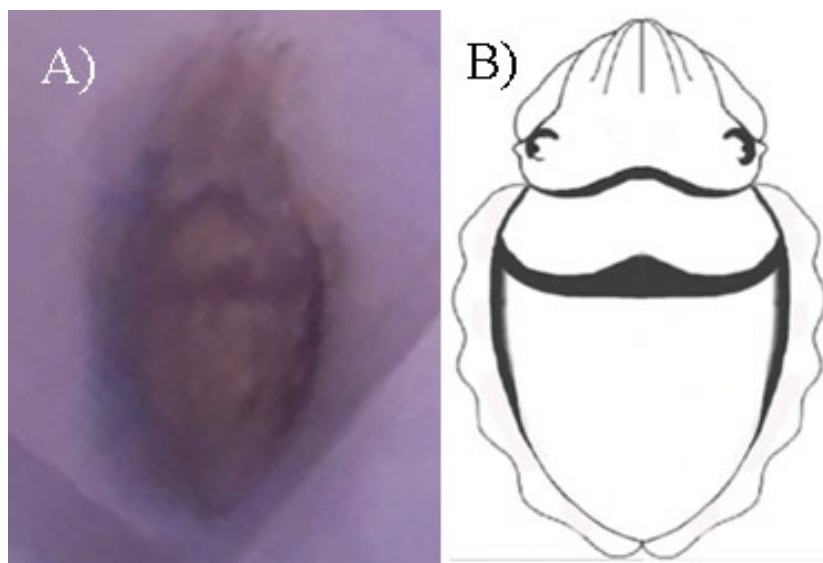


Figure 5.2: A) Cuttlefish displaying the directional banded pattern toward a conspecific [located on the right (not pictured)]. B) An illustrated example of the banded pattern (non-directional display), with darkened anterior transverse mantle line, prominent band just above the anterior transverse mantle line and the posterior head bar

Banded strike pattern

The 'Banded Strike' pattern also consists of a pale uniform background, with darkened anterior transverse mantle line, banded mantle bar, posterior head bar, in addition to the anterior head bar, eye rings, and banded arms (a previously undocumented chromatic component consisting of horizontal bands across the front arms) (Figure 5.2). The display was expressed four times in one trial (male-female) by a female cuttlefish. The pattern was always preceded by the Banded Display, with slight localisation of the banded arms nearest the conspecific, and succeeded with a retreat and display of weak zebra and/or eyespots. The pattern appears to be an aggressive display, as the pattern most commonly appeared before the female struck at the male. The male prompted this reaction twice, by attacking and continuing to strike after the female struck. Otherwise, the male displayed the intense zebra pattern before and after the female display. On the fourth occasion the Banded Strike flashed briefly while the male approached the Perspex barrier displaying the intense zebra display. Interestingly, on this occasion, the female adopted the banded face; directly facing the male and displaying a light uniform color on the mantle region.

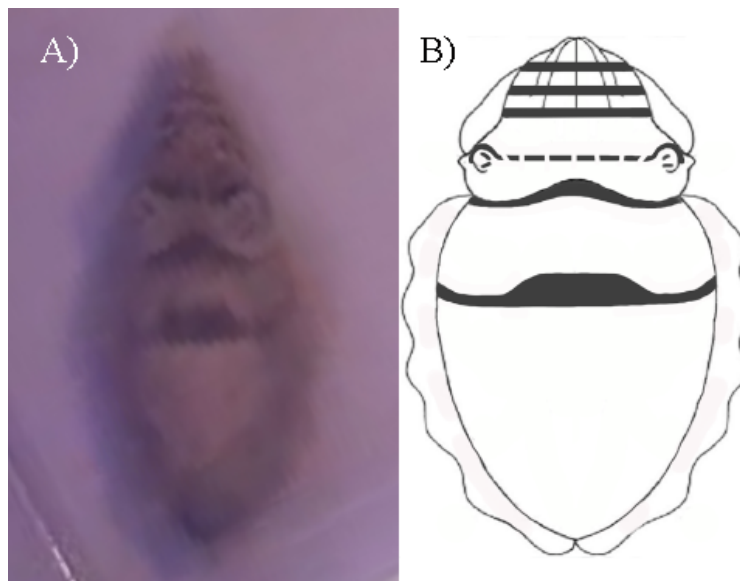


Figure 5.3: A) A female cuttlefish displaying the banded strike pattern at a male, just before striking. B) An illustrated example of the banded strike pattern, which darkens the anterior transverse mantle line, banded mantle bar, posterior head bar, in addition to the anterior head bar, eye rings, and banded arms (a previously undocumented chromatic component consisting of horizontal bands across the front arms)

5.4 Discussion

The coloration patterns and visual behavior of European cuttlefish are comparatively well known (Hanlon and Messenger 1988, Adamo and Hanlon 1996, Crook et al. 2002, Palmer 2006, Barbosa et al. 2008, Zylinski et al. 2009a), so it is perhaps surprising that this preliminary study of social interactions has revealed two new patterns, which along with Splotch pattern (which was found not to be expressed in this study) (Palmer et al. 2006) appear to form a related set of displays used by females in agonistic encounters with conspecifics. This may be because our experimental conditions are unusual. Studies of cuttlefish camouflage (Boal 1997/2006) have found that the size of the testing chamber alters behavior, and the current study utilizes smaller test chambers with animals that had been isolated for longer periods than previously. Similarly, the literature on social communication (Boal et al. 1999; Adamo and Hanlon 1996; Adamo et al. 2000) used cuttlefish that were housed socially. Sometimes they were separated at sexual maturity due to their aggressive behaviour, but still allowed to view one another through transparent dividers. Animals were sometimes isolated for two days before testing. Our cuttlefish were raised socially, but had been kept isolated after reaching sexual maturity, for six months. They were placed closer than they normally approach other cuttlefish - approximately one to three mantle lengths (Boal et al. 1999).

The Banded Strike pattern resembles the ‘Splotch’ body pattern in the head region (Palmer et al. 2006). Both patterns express the banded arms, darkened anterior mantle region, and light-brown posterior mantle region. The Splotch pattern differs in having white splotches and darker mottled spots distributed across the head and mantle regions. As far as we know the banded mantle bar and the banded face are unique to Banded Strike pattern, and these components that are not listed by Hanlon and Messenger (1988). The banded mantle bar may only be partial expression of the lower anterior mantle bar or increased expressed of the anterior transverse mantle line. Considering the spatial location and level of expression of the band compared to the anterior mantle bar or the anterior transverse mantle line, suggests the band is a new component.

From the limited evidence in this study, we speculate that the Banded Display pattern reduces agonistic encounters, whereas the Banded Strike directly precedes an attack to either sex. These descriptive and behavioral differences provide enough evidence to suggest the two patterns are distinct.

The visual characteristics of the Banded and Banded Strike patterns signal aggressive threats to a conspecific cuttlefish. The darkened bands on the mantle, head, and arms against a light background create strong contrast, making the cuttlefish more conspicuous (Figures 5.1 and 5.2). The transition from Banded to Banded Strike added more bands, with the armbands being the most prominent display.

While the Splotch pattern never appeared in our experiment, the visual and contextual similarities between the Splotch (Palmer et al. 2006), Banded Strike, and Banded displays suggests that these three patterns belong to a family of intraspecific communication signals, perhaps used in situations of conflict, but outside courtship. It may be that these three patterns can be used like the body patterns used in the presence of potential predators (Langridge 2007), whose expression is dependent upon the level of threat perceived (as measured by physical proximity to the threat). When a low-level threat appears, the cuttlefish express the eyespots without the dark fin line. The eyespot may appear unilaterally, on the mantle region closest to the perceived threat. When the threat continues to approach, the higher threat evokes the full deimatic display. Similarly, directional patterning was observed with the Banded pattern. When a conspecific startles the cuttlefish (e.g. attempt to escape, approach, or attack), it darkens the outer mantle region closest to the conspecific. The Banded Strike, like the full Deimatic pattern (used when the threat from a potential predator increases), was not expressed asymmetrically, this appears if the conspecific approached or displayed the Intense Zebra Display.

Directional patterning and context specific patterns have also been seen in cuttlefish responses to specific predators (Langridge 2007), and the intense zebra to males by males on spawning grounds. The Intense Zebra Display or zebra patterning intensifies on the mantle half closest to the conspecific. Amongst other cephalopod species, the Caribbean reef squid (*Sepioteuthis sepioidea*) display two separate patterns on either side of the body, known as the double signaling pattern (Byrne et al. 2003). During squid courtship, one half displays an agonistic pattern towards a rival male, while the other half displays a courting pattern toward the female. In the cuttlefish, this directional signaling is even more asymmetrical in the banded pattern. The pattern may express only on the front quarter region of the body or the full side of the body (Figure 5.3).

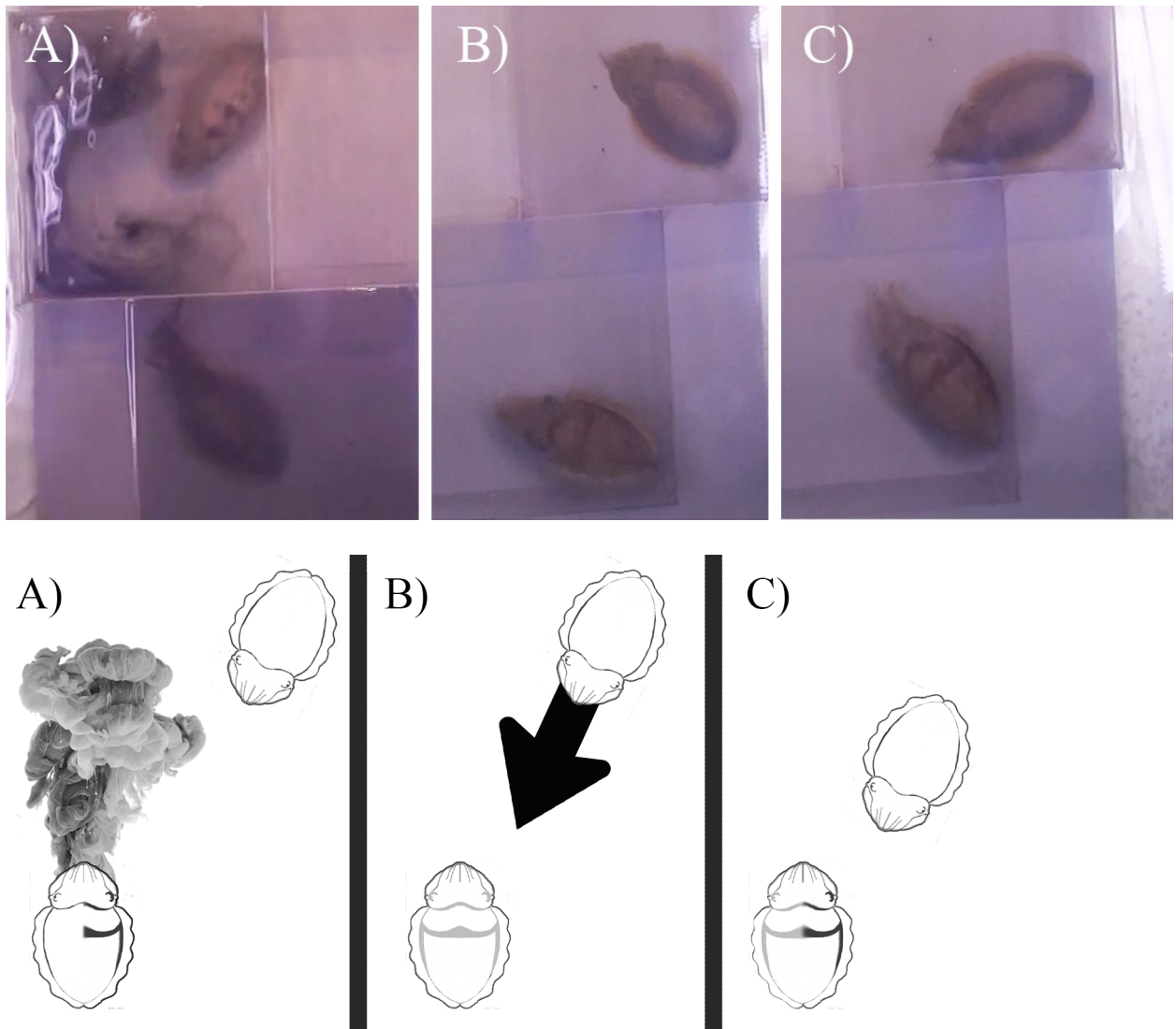


Figure 5.4: Three examples of directional signaling with the Banded display. A) Cuttlefish (upper) displays the Banded pattern after inking from an attack by the conspecific (below). B and C) The lower cuttlefish intensifies the Banded display in relation (outer mantle) when the proximity between the conspecific reduces.

In the female/female trial, the two cuttlefish both adopted a dark uniform pattern. In all other trials the light grey background elicited a light uniform pattern. Only in this instance

did both cuttlefish display the dark uniform pattern for the majority of the trial. Even more interesting, one female continually approached the other female's container and quickly alternated between light uniform and dark uniform, up to a one-minute interval. In several instances, this switching occurred several times per second, creating a strobe effect. After 10-minutes, the other female oriented itself toward the strobing cuttlefish and struck at it, causing it to ink (hence terminating the experiment). Because the effect was only observed once in the study, we cannot draw any larger conclusions about this novel behaviour, but only reported it due to its peculiarity.

To our knowledge, no previous study observed the Banded pattern or Banded Strike pattern (Hanlon and Messenger 1988; Boal 1997; Boal et al. 1999; Adamo et al. 2000; Palmer 2006). This may be due to the closer proximity than previous studies (Boal et al. 1999), individual variation due to the small number of subjects tested, or due to social isolation for several months before testing. The former studies examined the change between individual patterns and may have described the banded pattern to be a subset or partial display of another pattern. The development of the pattern repertoire in an individual cuttlefish depends upon the richness of the visual environment (Poirier et al. 2005). Larger numbers of cuttlefish and successful trials will be necessary to replicate the study with certainty that the pattern is expressed under similar conditions.

The intraspecific and interspecific displays demonstrate the specificity and diversity of cuttlefish communication. Broadening the conditions under which cuttlefish are tested may broaden our understanding of the cuttlefish's communicative capabilities in social and nonsocial contexts.

6. Discussion

In this thesis, we focused on cuttlefish (*Sepia officinalis*) visuo-motor body patterning, with respect to the underlying structure of the patterning system for camouflage and communication. Even though research into the patterning system has only been examined in great detail over past thirty years, the environmental and biological factors influencing pattern changes has been extensively examined. This thesis extends this body of knowledge through the use of advanced statistical techniques, in conjunction with previous methods used to quantify cuttlefish body patterns, providing new insights into the parameters of the body patterning system and how it relates to larger concepts in vision, camouflage, and communication.

Chromatic components are the building block of cuttlefish body patterns. Our exploration into how cuttlefish coordinate their expression has demonstrated that cuttlefish camouflage patterns do not possess discrete states, but suggests camouflage patterns possess a rich repertoire of available patterns that lie along a continuum. With such variability available for a cuttlefish to display, new opportunities arise to examine how environmental factors influence the subtle changes between states, perhaps providing important clues into how cuttlefish determine which pattern to display.

The cuttlefish rapid colour-changing system is as remarkable as it is unique and our investigations suggest cuttlefish continually fluctuate the range of patterns they display. It is interesting that the range of patterns expressed differs between individuals, yet they encompass a wider range of patterns at the population level. Even more interesting is that the number of patterns expressed depends on the spatial scale of objects on the substrate – one of the major environmental factors influencing larger ranges of pattern displays.

While our investigation into cuttlefish visual communication failed to discover any true visual language, we were able to distinguish language-like properties that demonstrate some structure to cuttlefish communication and inform us more about the information content. Through these experiments, we discovered two, previously undocumented, patterns used by female cuttlefish in agonistic encounters. Unfortunately, the pattern was observed for a small number of animals, but shows the potential that a richer repertoire of social patterns yet may be explored.

Implementing advanced statistical techniques, such as machine learning algorithms, provides the field with powerful tools that remove the subjective elements currently used to quantify and classify cuttlefish body patterns. More research is needed to discover which tools provide the best way to objectively quantify and classify the full extent of body patterns. The work in this thesis teases out the structure of the patterning system, however the clustering classification builds from handscoring techniques, which grades component expression by a human observer. There is a great deal of detail still to be determined in this colouration system of cuttlefish. This will involve creating objective methods to quantify component expression, through methods such as the scale-invariant Fourier transformation, allowing automatic classification of pattern components and their level of expression. Investigating these aspects of cuttlefish body patterning will allow us to ask questions about the underlying neural mechanisms guiding the visuo-motor system and how visual information influences it.

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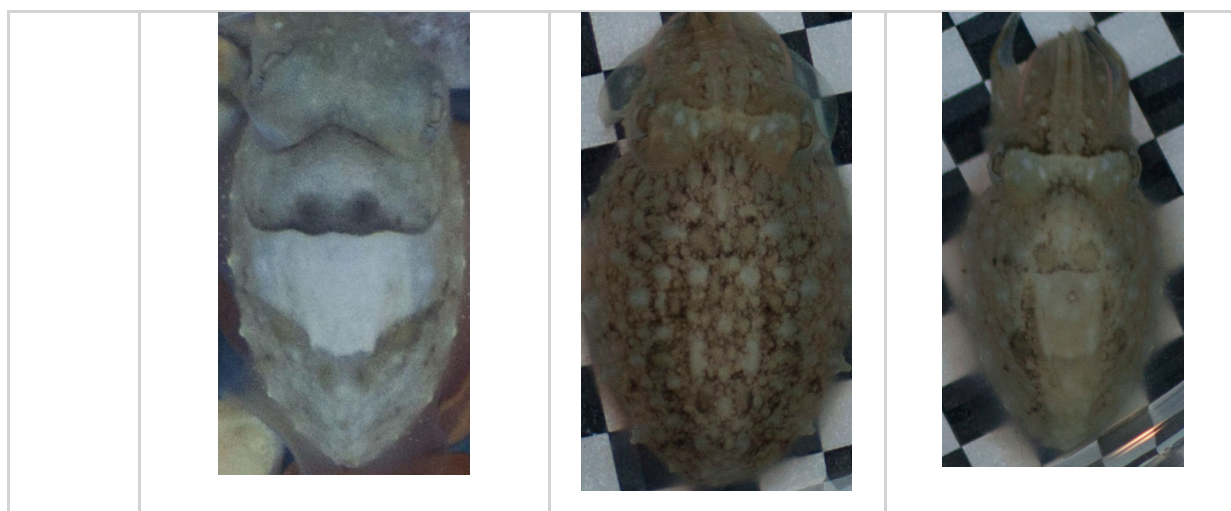
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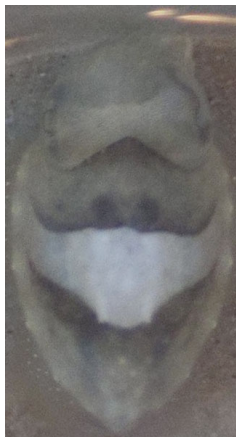


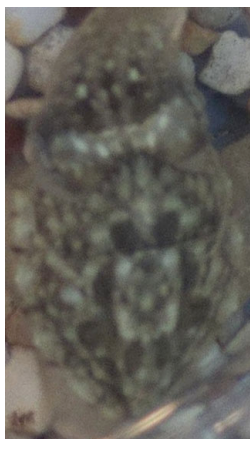
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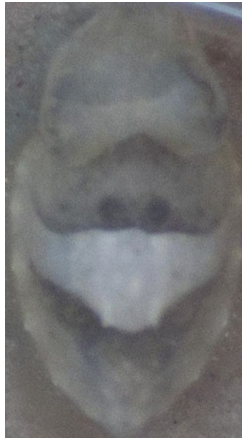






Appendix

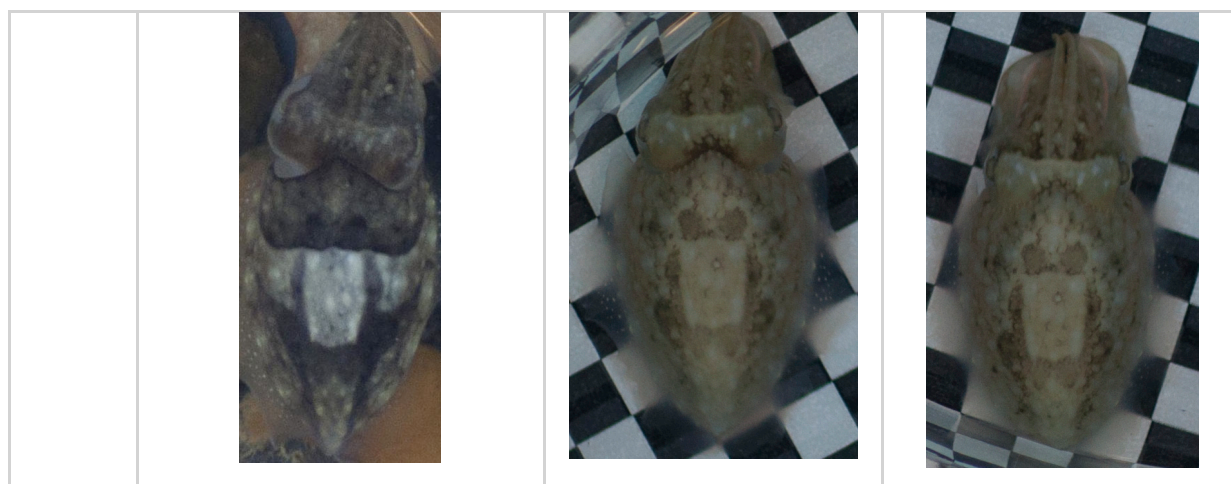
Cluster 1	Low (Furthest from Centroid (-))	Med (Closest to Centroid (0))	High (Furthest from Centroid (+))
PC1	3mm-140116-Run8-319 	Stone-130116-Run4-292 	Sand-070116-Run2-196 
PC2	12mm-221215-Run10-304 	8mm-140116-Run7-99 	Stone-130116-Run7-316 
PC3	Stone-120116-Run3-256 	8mm-140116-Run10-51 	8mm-241215-Run3-170 

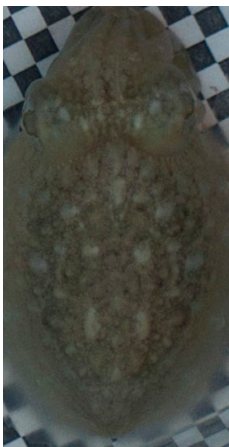







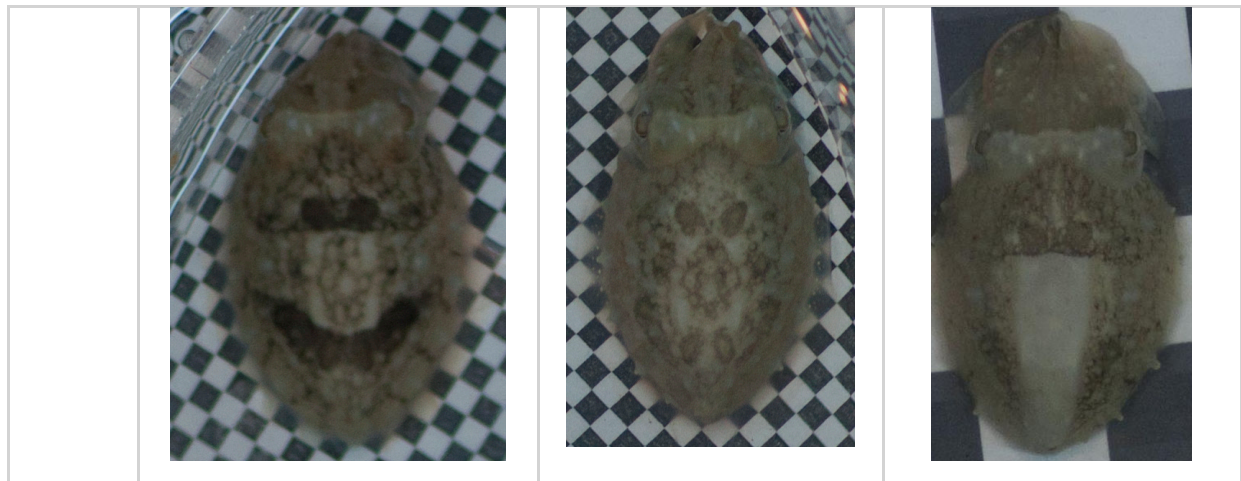
Cluster 2	Low (Furthest from Centroid (-))	Med (Closest to Centroid (0))	High (Furthest from Centroid(+))
PC1	12mm-161215-Run5-4 	Peb-110116-Run8-76 	Sand-070116-Run2-88 
PC2	12mm-141215-Run3-52 	3mm-040116-Run1-88 	Peb-100116-Run2-220 
PC3	Stone-130116-Run6-100	Sand-070116-Run6-112	12mm-140116-Run6-136

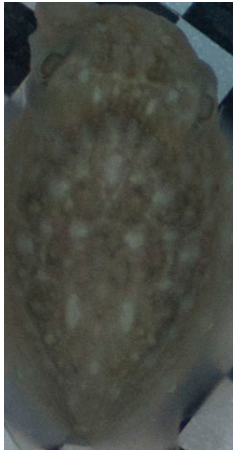
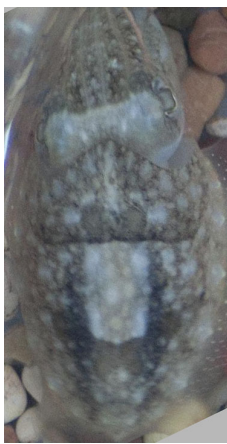
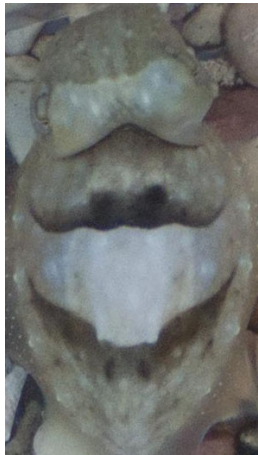


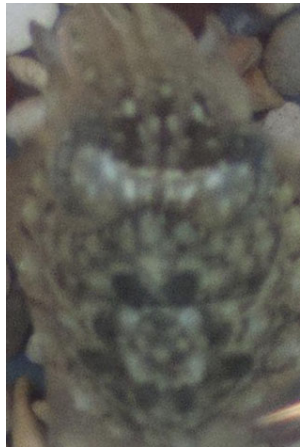


Cluster 3	Low (Furthest from Centroid (-))	Med (Closest to Centroid (0))	High (Furthest from Centroid(+))
PC1	3mm-140116-Run7-31 	3mm-050116-Run10-50 	Sand-070116-Run2-76 
PC2	12mm-141215-Run2-16 	3mm-140116-Run7-247 	8mm-271215-Run6-207 
PC3	Stone-120116-Run1-183 	8mm-241215-Run3-314 	8mm-241215-Run3-290 




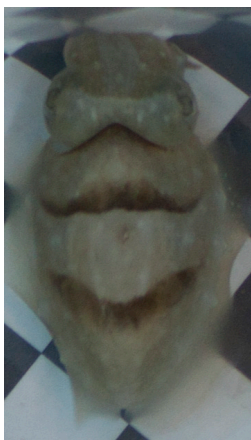
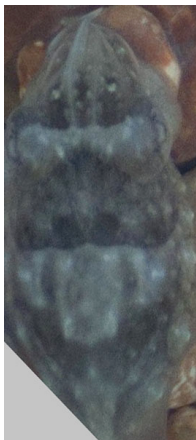
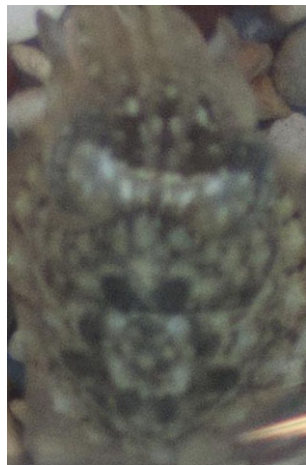


Cluster 4	Low (Furthest from Centroid (-))	Med (Closest to Centroid (0))	High (Furthest from Centroid(+))
PC1	3mm-140116-Run7-235 	Stone-120116-Run3-124 	Peb-110116-Run10-4 
	12mm-141215-Run3-4 	Sand-080116-Run9-160 	8mm-271215-Run6-135 
PC3	3mm-040116-Run2-278	3mm-050116-Run10-319	12mm-181215-Run8-28



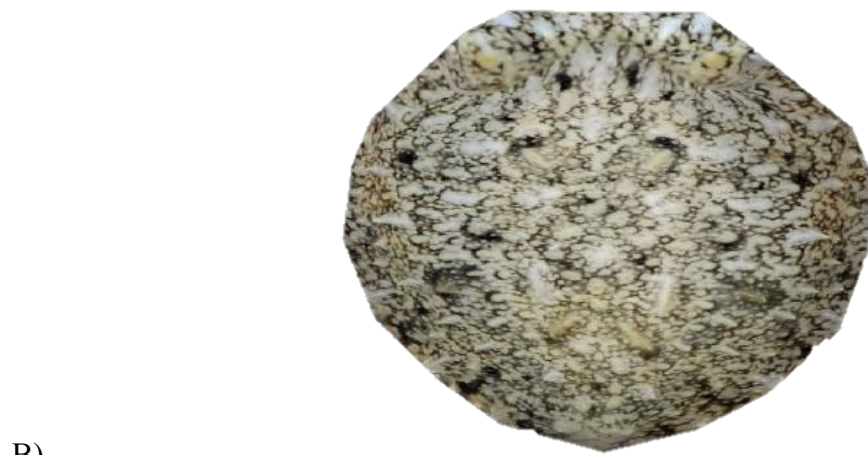
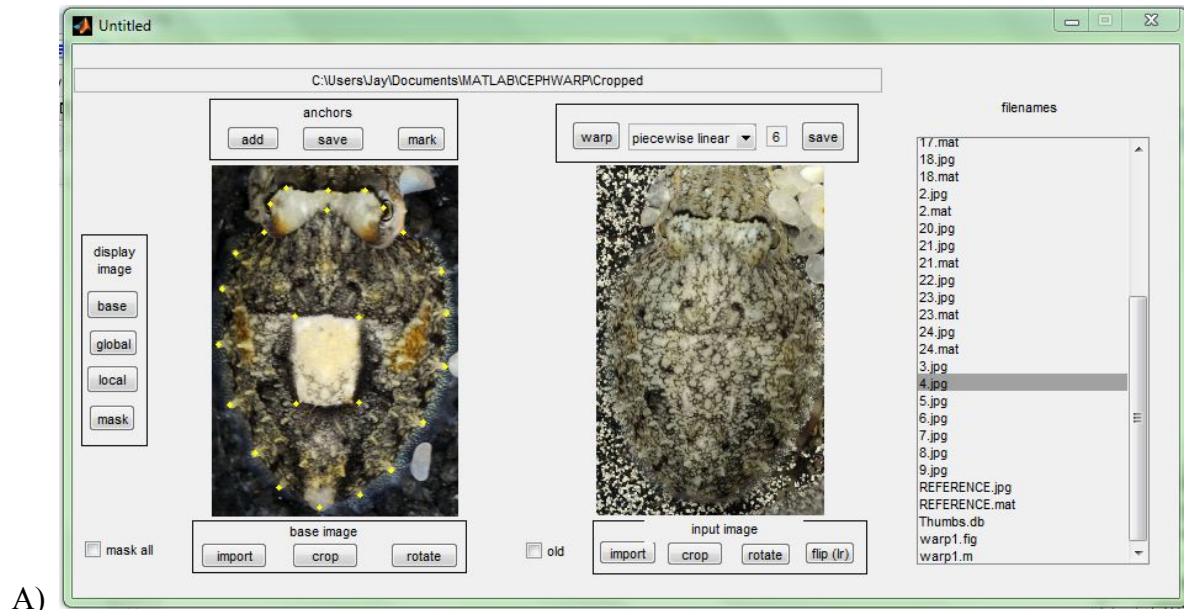
Cluster 5	Low (Furthest from Centroid (-))	Med (Closest to Centroid (0))	High (Furthest from Centroid(+))
PC1	8mm-271215-Run4-163 	Peb-110116-Run9-16 	Peb-140116-Run6-184 
PC2	12mm-181215-Run8-340 	12mm-141215-Run2-88 	Peb-100116-Run2-232 
PC3	8mm-271215-Run4-31	12mm-181215-Run8-268	12mm-181215-Run8-16



Cluster 6	Low (Furthest from Centroid (-))	Med (Closest to Centroid (0))	High (Furthest from Centroid(+))
PC1	12mm-161215-Run5-76 	12mm-141215-Run2-52 	Sand-070116-Run2-172 
PC2	12mm-141215-Run2-4 	Stone-130116-Run9-352 	Peb-100116-Run2-232 
PC3	Stone-120116-Run1-87	Peb-100116-Run3-352	12mm-181215-Run8-4



Appendix 1: The 'up' and 'across' variation along each principal component axis for the six clusters generated. The patterns with the lowest (-) and highest (+) values on a given pc axis, as well as, the pattern closest the centroid are represented.



Appendix 2: A) The Warping software (Anderson et al. 2003). The image on the left serves as the reference, with the 25 yellow dots placed along the head, mantle, and white square. The image on the right is the warping image, where the yellow dots are placed accordingly to the same location as the reference image. B) An example of a warped image.